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INFLUENCE OF COLD STORAGE ON THE VIABILITY OF VEDALIA BEETLE, *Rodolia cardinalis* (MULSANT) (COLEOPTERA: COCCINELLIDAE)

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ABSTRACT

The vedalia predator *Rodolia cardinalis* considered as a main natural enemy for the biological control of mealy bugs, *Icerya purchasi*, *I. aegyptiaca* and *I. seychellarum*. Laboratory studies were conducted to determine the effect of cold storage on the developmental stages of *R. cardinalis*. Eggs of the predator were stored for 5, 10, 15 and 20 days at 6, 10 and 14 °C, , larval instars were stored for 5, 10, 20 and 30 days at 10 and 14 °C. *R. cardinalis* pupae were stored 5, 10, 20, 30 and 40 days at 6, 10 and 14 °C, while *adults* were stored for 5, 10, 20 and 30 days at 6 and 10 °C. Hatchability percentage of eggs decrease with the increase of storage period at 6°C, it averaged 84 ±10.2 (eggs one day old) and 78 ± 11.6 (eggs two days old) after 5 day of storage at 6°C, however at 6°C and 10°C eggs one and two days old, no egg hatching was observed after 25 days of storage. All eggs held for 25 days at 6°C and 10°C failed to hatch. The survival of third and fourth instar larvae stored at 6°C and 10°C was higher than the first and second instar. The fourth instar larvae of *R. cardinalis* were the most tolerable for cold storage. These results also provide novel findings that the fourth larval instar and eggs of *R. cardinalis* can be stored for twenty days at 10 °C, adult and pupae stored at 6 °C for twenty days with no reduction in viability for each stage. The results indicate that a cold storage of *R. cardinalis* could be used for maintaining and accumulating these predators during mass propagation for release in a biological control program and increasing the shelf-life of predators in clean agriculture.

Keywords*: Rodolia cardinalis* , mealy bugs, cold storage, biological control.

INTRODUCTION

Vedalia beetle, *Rodolia cardinalis* (Mulsant) (Coleoptera: Coccinellidae), has been a primary natural enemy regulating populations of cottony cushion scale, *Icerya purchasi* Maskell (Homoptera: Margarodidae) in California since it was introduced in the winter of 1888–1889 from Australia (Caltagirone and Doutt, 1989; Doutt,1964). It provides excellent biological control of cottony cushion scale because of its high reproduction rate, rapid development, and host specificity (Quezada and DeBach, 1973).

Storage of natural enemies assure their availability in sufficient number at the time of release. Therefore, the development of storage techniques for biocontrol 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; Ravensberg, 1992).

Storage techniques must ensure the availability of natural enemies quality (Bigler, 1994). Integration of cold storage of predaceous insects with mass rearing of them, could help in achieving the main purpose of biological control (Abdel-Salam, 2001). Long-term storage could aid in the cost-effective mass production of beneficial insects. Pre-conditioning, insect developmental stage and environmental conditions should be considered when selecting storage conditions in order to obtain the highest performance after storage (Coudron et al., 2oo7). Efficient storage of this biological control agent could improve its current production and use. Cold storage can permit a cost-effective production schedule, providing a means to conserve biological control agents when not immediately needed (Pitcher et al. 2002, Ayvaz *et al*. 2008, Kui *et al.* 2014). The purpose of the proposed project was to determine the effect of cold storage on the aviability of different stages of vedalia beetle, *Rodolia cardinalis* .

MATERIALS AND METHODS

Cold storage of egg stage.

A laboratory culture of *R. cardinalis*, was initiated by collecting its pupae from *Ficus nitida* trees free from any insecticide application. Collected pupae were placed in petri dishes at 25 ±1 °C and 70 ± 5 % R.H. in an incubator till adult emergence, then adults were fed on *I. seychellarum*. Newly deposited predator eggs were daily collected. Eggs of each one day and two days old were divided into three groups, each group consisted of 250 eggs. The egg groups were stored at 6, 10 and 14 °C, respectively while the photoperiod was 16:8 L:D. At five days intervals, 50 eggs of each group (10 eggs/ petri-dish, and replicated five times), were transferred gradually from the storage temperature (6, 10, 14°C) and incubated at 25°C. Therefore percentage of hatchability were observed and recorded. This procedure was maintained after 10, 15 and 20 days from storage.

Cold storage of larval stage

Five larvae of the predator were placed in petri-dish, and replicated five times for each instar. The larvae were held at two constant temperature 6°C and 10 °C and 16:8 L:D. After the storage periods (5, 10, 20 and 30 days), all larval instars were transferred to an incubator at 25°C and fed on *I. seychellarum*. Moreover, survival percentage and percentage of larvae transformed to the next instar were calculated and recorded until pupation.

Cold storage of pupal stage

Newly pupae of *R. cardinalis* were placed at 25°C for one day, then transferred to incubators that held at 6, 10, 14 °C and 70 ± 5 % R.H. with L:D 16:8 for 5, 10, 20, 30 and 40 days. Twenty pupae were held at each temperature and storage period. With cach of storage time, the pupae were transferred gradually to 25°C, 70 ± 5 % R.H. and L:D 16:8. Thus, pupal duration, adult emergence percentage was recorded. Immediately after adult emergence, adults were reared on *I. seychellarum* in order to determine their fecundity, longevity and survival.

Cold storage of adult stage

A laboratory culture of *R. cardinalis*, were initiated from pupae collected from Ficus *nitida* trees free from insecticides. Since the pupal stage was the most abundant stage, individuals of the pupal stage were collected and placed in petri-dishes at 25°C and 70 ± 5 % R.H. in an incubator till adult emergence and fed on *I. seychellarum.* Newly emerged adult of *R. cardinalis* fed on *I. seychellarum* for one day at 25°C, were transferred to incubators held at 6, 10°C, 70 ± 5 % R.H. and L:D 16:8 for 5, 10, 20, 30 days and feded on *I. seychellarum* . Twenty adults held at each temperature and storage period, were transferred to 25°C, 70 ± 5 % R.H. and L: D 16:8 and reared on *I. seychellaru.* Percentage of survival fecundity and longevity were determined.

Statistical analysis :

The obtained data was statistically analyzed by using one way ANOVA; (Cosstat, 1990).

RESULTS AND DISCUSSION

Cold storage of egg stage.

1. Storage of newly deposited eggs.

Results in Table 1 show, the % of hatchability decrease with the increase of storage period at 6°C, it averaged 84 ±10.2, 76 ± 4.8, 74 ± 10.12 and 14 ± 12.6 after 5,10,15 and 20 days of storage, respectively. There was a significant difference in % of hatchability resulting from eggs stored for 5, 10, 15 and 20 days. At 10°C the % of hatchability were 86 ± 4.8, 94 ± 4.8, 82 ± 7.48 and 90 ± 0.6 for storage periods of 5, 10, 15 and 20 days, respectively. No difference between % of hatchability within storage periods. Meanwhile at 14°C, % of hatchability was 94 ± 0.8, 82 ± 16 and 80 ± 4 % for storage periods 5,10 and 15 days, respectively. Eggs storage at 14°C for 20 days hatched during storage period.

2. Cold storage of two days deposited eggs.

Results in Table 1, show that the % of hatchability at 6°C were 78 ± 11.6, 80 ± 6.32, 70 ± 8.9 and 44 ± 12.6 for storage periods 5, 10, 15 and 20 days, respectively. On the other hand at 10°C the % of hatchability were 96 ± 4.8, 84 ± 0.8, 94 ± 0.8 and 92 ± 7.4 for eggs stored for 5,10,15 and 20 days, respectively. There were no significant differences among % of hatchability within storage periods at 10 °C (Table 1). Regarding to 14°C, % of hatchability were 74 ± 10.2, 86 ± 8.0 and 83 ± 5 % for storage periods 5,10 and 15 days, respectively. These data indicated that, long term cold storage at 10°C lead to significant reduction in percentages of hatchability. In conclusion, it is clear that eggs (two days old) can be stored for 20 days with 92% hatchability. These results disagreed with Abdel-Salam and Abdel-Baky (2000), noted that 65% of *C. undecimpunctata* eggs (7 days stored at 6°C) hatched, meanwhile eggs resulting from a storage period of 15 days did not hatch. These results came in the same line with Montgomery *et al.,* (2002) who noted that the hatchbility of *Scymnus ningshanensis* eggsstored at 6°C for two week not affected , but longer storage reduced percent hatched of eggs and was zero after 5 week of storage. Eggs of *Eriopis connexa* (Coccinellidae) could not kept for more than one day at 4°C without incurring 30% mortality; after 14 days mortality was 83% (Miller, 1995).

Table (1): Effect of cold storage on hatchability percent of *R. cardinalis* eggs.

Period from ovi.	Storage periods	Hatchability (%)			
		Temperature regimes			L.S.D
		6ºC	10ºC	14ºC	
One days old	5 days	84 ± 10.2 a *ab*	86 ± 4.8 a abc	94 ± 0.8 a *b*	17.2790
	10 days	76 ± 4.8 a *ab*	94 ± 4.8 a *ab*	82 ± 16 a *ab*	21.7432
	15 days	74 ± 10.2 a *b*	82 ± 7.48 a *c*	80 ± 4 *ab*	21.2213
	20 days	14 ± 10.2 a *d*	90 ± 0.6 b *abc*	-----	20.1323
Two days old	5 days	78 ± 11.6 b *ab*	96 ± 4.8 a *a*	74 ± 10.2 b *a*	20.2615
	10 days	80 ± 6.32 a *ab*	84 ± 0.8 a *bc*	86 ± 8 a *ab*	16.1631
	15 days	70 ± 8.9 b *b*	94 ± 0.8 a *ab*	83 ± 5 *ab*	20.1323
	20 days	44 ± 12.6 a *c*	92 ± 7.4 a *abc*	-----	15.327
Control		93.6 ± 6.34 *a*	93.6 ± 6.34 *abc*	93.6 ± 6.34 *ab*	
LSD		14.328	9.49625	15.9814	----------

Means followed by the same letter in a row or the same italic letter in column are not significantly differences at 5% level of probability (Duncan's Multiple RangeTest)

Effect of cold storage and storage periods on survival rate of Rodolia cardinalis larvae.

1. Storage at 6°C.

Data presented in Tables (2 & 3) show that, percentages of larval survival four 1st were 90 ± 10, 80 ± 14.4 and 0.0 % for storage periods 5, 10 and 20 days, respectively and 75 ± 8.6, 30 ± 10 and 0.0% of these larvae reached to adult stage after storage periods of 5, 10 and 20 days, respectively in comparison with 70 ± 10 for control (unsorted) (table 3). Survival rate of second larval instars recorded 100, 45 ± 8.6 and 0.0% after 5, 10 and 20 days of storage and 85 ± 8.6, 45 ± 8.6 and 0.0 of these larvae reaching to adult after the same periods of storage, respectively. Whereas survival rate of third and fourth instar larvae was close to 100 % for five days of storage, meanwhile after 10 and 20 days of storage survival were 90 ± 17.3 and 95 ± 8.6, 35 ± 25.9 and 90 ± 10.0 for third and fourth instar larvae, respectively. Third larval instars storage for 20 days failed to reach adult stage, meanwhile 85 ± 8.6 of fourth larval instar stored for 20 days, reached to adult stage. The survival rate of third and fourth instar larvae was 0.0 after 30 days of storage.

2. Storage at 10°C.

Data in Tables 2 & 3 show that, survival of the four larval instar of *R. cardinalis* recorded 100% after five days and 90 ± 10, 100, 100, 100 and 100% of these larvae succeeded reach to adult stage, whereas after 10 days of storage, survival lasted 90 ± 10, 80 ± 5.0 , 100 ± 0.0 and 100 ± 0.0 % for the four larval instars, respectively and 65± 16.5, 60 ±. 14, 100 and 100% of these larvae reached to adult stage. Meanwhile the survival percentage of the four larval instars after 20 days of storage recorded 0.0, 0.0, 85 ± 8.6 and

100 %, respectively. When first, second and third instar larvae held at 10 °C for 30 days survival percentages were 0 %, meanwhile the survival of fourth instar larvae recorded 95 ± 8.6 % from the same period of storage and 45 ± 8.6 of these larvae reached to adult stage. It can be concluded however, that the fourth larval instar can be stored for 20 days at 10 °C safely with no effect on survival and adult emergence.

After 15 days of storage *C. undecimpunctata* larvae lasted 0, 10, 15 and 15% for first, second, third and fourth larval instar at 6°C and no larvae survival after 30 days of storage (Abdel-Salam and Abdel-Baky ,2000). These results are in agreement with (Gagne and Coderre 2001) on the coccinellid *Coleomegilla maculata* lengi . Survival was close to 100% for the first two weeks of storage, but decreased drastically afterward and was 0% after 5 weeks, when the larvae returned to 24°C.

Table (2): Effect of cold storage (6Cº and 10Cº) and storage periods on survival of *R. cardinalis* larvae.

Temperature	Larval instars	*Storage periods (days)*				Check	LSD
		5	10	20	30		
6Cº	First	90 ± 10 ab	80 ± 14.14 b	0.0 c	0.0 c	100 a	13.48
	Second	100 ± 0.0 a	45 ± 8.6 b	0.0 c	0.0 c	100 a	7.78
	Third	100 ± 0.0 a	90 ± 17.3 a	35 ± 25.9 b	0.0 c	100 a	21.66
	Fourth	100 ± 0.0 a	95 ± 8.6 a	90 ± 10 b	0.0 c	100 a	10.29
10Cº	First	100 ± 0.0 a	90 ± 10 b	0.0 c	0.0 c	100 a	7.78
	Second	100 ± 0.0 a	80 ± 0.0b	0.0 c	0.0 c	100 a	7.78
	Third	100 ± 0.0 a	100 ± 0.0 a	85 ± 8.6 b	0.0 c	100 a	6.74
	Fourth	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a	95 ± 8.6 a	100 a	6.74

Means followed by the same small letter in a row are not significantly differences at the 5% level of probability (Duncan's Multiple Range Test).

Table (3): Effect of low temperature (6Cº and 10Cº) and storage periods on adult emergence from the stored larvae of *R. cardinalis*.

Temperature	Larval instars	Storage periods (days)				Check	LSD
		5	10	20	30		
6Cº	First	75 ± 8.6 a	30 ± 10 b	0.0 c	0.0 c	70 ± 10. 8 a	12.9
	Second	85 ± 8.6 a	45 ± 8.6 b	0.0 c	0.0 c	85 ± 8.6 a	11.67
	Third	100 ± 0.0 a	90 ± 10 a	0.0 b	0.0 c	100 a	10.29
	Fourth	100 ± 0.0 a	90 ± 10.3 ab	85 ± 8.6 b	0.0 c	100 a	10.92
10Cº	First	90 ± 10 a	65 ± 16.5 b	0.0 c	0.0 c	70 ± 10 8 b	12.96
	Second	100 ± 0.0 a	60 ± 14 c	0.0 d	0.0 d	85 ± 8.6 b	12.90
	Third	100 ± 0.0 a	100 ± 0.0 a	75 ± 8.6 b	0.0 c	100 a	6.74
	Fourth	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a	45 ± 8.6 b	100 a	7.78

Means followed by the same small letter in a column are not significantly differences at the 5% level of probability (Duncan's Multiple Range Test).

Effect of low temperatures and storage periods on biological characters of pupal stage of *R. cardinalis*

1. Storage at (6°C)

Data presented in Table 4 show the biological characters of adult emerged from pupae subjected to 6 °C for different periods. Regarding adult emergence, there is no difference of this ratio when pupae stored for 5 days compared with check treatment,

When the adult emergence reached 100%. Meanwhile, adult emergence decreased gradually with increasing the storage periods in comparison with check. Percentage of adult emergence reached 90 ± 12.24, 80 ± 18.7, 45 ± 18.7 and 30 ± 2.9% when pupae were stored for 10, 20, 30 and 40 days, respectively. Increasing the storage periods for (40-days) led to malformed adults, whereas no egg oviposition was noted. Significant differences were found among percentage of adult emergence from stored pupae for 5, 10, 20, 30 and 40 days and check (unstored), but there was insignificant differences among control, storage periods for 5, 10 and 20 days. Also there was insignificant differences between 30 and 40 days. The pre-oviposition of female resulting from storage conditions were 3.4, 3.6, 4,4 and 7.4 days for control, storage periods of 5, 10, 20 and 30 days, respectively. Oviposition periods of females emerged from pupae subjected to 6 °C for 5, 10, 20 and 30 days were 31.6, 26, 30 and 26.2 days in comparison with 33.8 days / a control female (unstored). There was an insignificant impact on the oviposition period of female between any treatments.

The fecundity of females resulting from pupae subjected to storage condition were 127.4, 96.8, 81.8 and 20.4 eggs for storage periods of 5, 10, 20 and 30 days, while for control was 255 egg. There was a significant impact between the control and other treatments, meanwhile there was no significant effect on the fecundity of female resulting from pupae stored for periods 5, 10 and 20 days, respectively.. The male longevity were 31 ± 7.12, 18.4± 1.6, 18.2 ± 5.2, 17.8 ± 5.34, 12 ± 5.2 and 3.5 ± 0.7 days for control, storage periods 5, 10, 20, 30 and 40 days, respectively. There were significant differences between control and storage periods for 5, 10, 20, 30 and 40 days of storage on male longevity.

2. Storage at 10°C.

Data in Table (5) shows that the % of adult emergence from pupae stored at 10°C was 95 ± 5, 100, 90 ± 20 and 85 ± 10 for storage periods of 5, 10, 20 and 30 days, respectively. The duration of the pupal stage decreased as the storage period increase from 5 to 30 days, it was 5 ± 0.0, 3.6 ± 1.01, 2.4 ± 0.48 and 0.8 ± 0.48 days for storage period 5, 10, 20 and 30 day, respectively compared with 7.6 ± o.48 days for control. There were significant differences between pupal stage in the control and in the storage periods. The pre-oviposition of female resulting from storage conditions were 4.8 ± 0.4, 5.6 ± 7.85, 5.8 ± 1.46 and 5.8 ± 7.6 days for storage periods 5, 10, 20 and 30 days, respectively. There were insignificant differences for pre-oviposition between storage periods. The oviposition periods were 30.2 ± 7.33, 19.8 ± 10.8, 21.4 ± 10.3 and 10.4 ± 3.38 resulting from pupae stored at 10 °C for 5, 10, 20 and 30 days compared with 33.8 ± 5.03 days for control (unstored). There was insignificant impact on the oviposition period of female between any treatments except between 30 days stored pupae and untreated control. The female longevity did not differ at storage period 5 to 20 day in comparison with check, it lasted 38 ± 6.5, 31.8 ± 7.9, 32.8 ± 12.8 and 18.4 ± 3.2 days for female emerging from pupae subjected 10 °C for 5, 10, 20 and 30 days opposed to 40 ± 4.24 days / a control female (unstored). The fecundity of female decrease as the storage period increase, it were 114 ± 35.13, 78.6 ± 42.1, 60.2 ± 22.8 and 17.8 ± 2.3 eggs resulting from pupae stored for 5, 10, 20 and 30 day, respectively in comparison with 255 ± 50.4 eggs for control (unstored). Male longevity resulting from pupae held at 10 °C for 5, 10, 20 and 30 days lasted 25.6 ± 7, 24.8 ± 5.5, 27.6 ± 10.2 and 15.6 ± 2.8 days compared to 31 ± 7.12 days for control (unsorted).

3. Storage at 14°C.

Data presented in Table 6 shows that the % of adult emerging from pupae stored for 5, 10 and 20 days were 100 ± 0.0, 95 ± 10 and 90 ± 10 %, respectively. Pupal duration decreases with increasing storage periods. The averaged pupal stage durations were 6.4 ± 0.48 for storage period 5 day, 3.2 ± 0.4 for storage period 10 day and 0.2 ± 0.4 for storage period 20 day. The averages of pre-oviposition period were 6.2 ± 0.4, 3.2 ± 0.4 and 9.2 ± 1.16 days resulting from pupae stored for 5, 10 and 20 days, respectively. Female longevity (mean ± SE) were 44 ± 3.4, 20.6 ± 5.31 and 26.8 ± 1.93 days resulting from pupae stored for 5, 10 and 20 day, respectively. The average fecundity of females resulting from pupae stored for 5, 10 and 20 day were 62.2 ± 6.33, 34.5 ± 0.3 and 34.5 ± 14.9 eggs laid by female. There were significant variation on fecundity between all treatments in comparison with control. Male longevity resulting from pupae stored for 5, 10 and 20 days were 35.2 ± 3.86, 16.6 ± 3.16 and 21.2 ± 2.31 days for storage periods 5, 10 and 20 days, respectively.

These finding are in general agreement with those obtained by Abdel-Salam and Abdel-Baky, 2000, they found that, emergence of *C. undecimpunctata* adults lasted 85.5, 65, 25 and 0% when the pupal stage stored for 7, 15, 30 and 45 days at 6°C. In another study, there is no mortality % of adults when the pupae of *Eriopis connexa* stored at 4°C for three weeks but the adults mortality reached 100% after 7 weeks of storage (Miller, 1995), these was in agreement with our results which indicated that the period of pupal storage affected significant with longest the period of storage. A temperature of 6 ± 1°C was suitable for keeping pupae and adults of *Chilocorus bijugus* in the laboratory during winter months for up to 43 and 110 days, respectively, prior to field release during summer month (Rawat *et al.,* 1992).

Table (6): Effect of different storage periods on pupal stage and biological characters of *R. cardinalis* adults emerged from pupae stored at 14Cº.

Storage Periods (days)	Adult emergence (%)	Duration in days ±SE						Fecundity/ female
		Pupal stage after treatment	Pre-oviposition.	Oviposition	Post-oviposition	Female longivity	Male longevity	
5	100 a	6.4 ± 0.48 a	6.2 ± 0.4 b	34± 3.43 a	3.8 ± 0.4 a	44 ± 3.34 a	35.2 ± 3.86 a	62.2 ± 6.33 b
10	95 ± 10 a	3.2 ± 0.4 b	3.2 ± 0.4 c	14.4 ± 5.4 b	3 ± 0.6 ab	20.6 ± 5.31 c	16.6 ± 3.61 b	34.5 ± 0.3 c
20	90 ± 10 a	0.2 ± 0.4 c	9.2 ± 1.16 a	14.4 ± 2.05 b	2.2 ± 0.4 ab	26.8 ± 1.93 bc	21.2 ± 2.31 b	34.5 ± 14.9 c
Control (unstored)	100 a	7.6 ± 0.48 a	3.4 ± 1.02 c	33.8 ± 5.03 a	2.8 ± 0.74 b	40 ± 4.24 b	31 ± 7.12 a	255 ± 50.4 a
LSD	16.75	2.92	1.14	6.281	0.874	7.44	6.77	17.34

Means followed by the same small letter in a column are not significantly differences at the 5% level of probability (Duncan's Multiple Range Test)

Effect of low temperature and storage periods on adults of *R. cardinalis*

1. Storage at 6°C.

From the results in Table 7, it can be seen that the survival of adult resulting from storage periods of 5, 10, 20 and 30 days were 100 ± 0.0, 100 ± 0.0, 100 ± 0.0 and 50 ± 31.6 %. The pre-oviposition periods resulting from storage conditions of 5, 10, 20 and 30 day were 4.8 ± 0.4, 3.8 ± 0.74, 4.8 ± 0.74 and 8.6 ± 0.8 days, respectively. Meanwhile the oviposition periods were 18.2 ± 1.46, 22 ± 2.28, 19.6 ± 1.85 and 4 ± 0.63 for storage periods of 5, 10, 20 and 30 days, respectively. There were significant differences between oviposition periods and check. Results show that after stored for 5, 10, 20 and 30 days a female produced an average of 69.8 ± 6.93, 79.2 ± 16.4, 42 ± 8.06 and 15.4 ± 1.8 eggs opposed to 255 ± 50.4 eggs resulted/ a control female (unstored). Data indicate that there were significant differences in fecundity among female that storage for 5 to 30 day and check, but no significant impact was noted for storage period 5 and 10 days. Female longevity were 25.6 ± 1.35, 31.8 ± 5.41, 36.2 ± 2.48 and 25.6 ± 2.05 for storage periods 5, 10, 20 and 30 days, respectively. Male longevity were 21.4 ± 1.95, 22.6 ± 2.8, 24.6 ± 2.41 and 17.22 ± 1.16 days for different storage periods, respectively in comparison with 31 ± 7.12 days for control (unstored) . There were not significant differences between male longevity resulting from storage periods.

2. Storage at 10°C.

Survival of adult stored for 5, 10, 20, and 30 days at 10 °C. were 100, 100, 80± 10 and 80 ± 18%, respectively (Table 8). The pre-oviposition periods were 3 ±0.63, 3 ± 0.63, 5.2 ± 3.48 and 4 ± 0.63 days for storage conditions. Oviposition periods were 21.6 ± 3.13, 20.4 ± 7.2, 19 ± 11.36 and 13.6 ± 2.05 for storage periods 5, 10, 20, and 30 days, respectively. The female longevity were 27.4 ± 4.95, 27.4 ± 7.19, 39.6 ± 9.85 and 46.4 ± 3.72 days, meanwhile male longevity were 12.6 ± 2.24, 18 ± 2.89, 14.8 ± 3.8 and

35.4 ± 5.71 days for storage periods 5, 10, 20, and 30 days, respectively. Fecundity of female storage for 5, 10, 20, and 30 days were 92.8, 52.4, 74.2 and 11.2 eggs per female, respectively. There were significant differences in oviposition periods and number of eggs for the different periods of storage and check.

Figure 19 shows that storage periods and low temperatures had no effect on female longevity in comparison with check. Figure 20 shows that, storage periods and low temperatures affected greatly on the female fecundity in comparison with check. Moreover, female fecundity varied according to storage temperatures and storage periods.These results disagreement with Rawat *et al.,* 1992, they recorded temperature of 6°C ± 1 was suitable for keeping pupae and adults of *Chilocorus bijugu*s in the laboratory during winter months up to 43 and 110 days respectively, prior to field release during summer months. In another case these results are in agreement with Yigit et al., 1994, they mentioned, adult of *Cryptolaemus montrouzieri* and *Nephus includens* could be stored for longer periods at 15 than at 7°C. Our finding came in the same line with (Umberto et al., 2008).

Table (7): Effect of different storage periods on the biological characters of *R. cardinalis* adult stored at 6Cº.

Storage Periods (days)	Survival of adult from storage period	Duration in days ±SE					
		Pre-oviposition	Oviposition	Post-oviposition	Female longevity	Male longevity	Fecundity/ female
5	100 a	4.8 ± 0.4 b	18.2 ± 1.46 b	2.6 ± 0.48 c	25.6 ± 1.35 c	21.4 ± 1.95 bc	69.8 ± 6.93 b
10	100 a	3.8 ± 0.74 bc	22 ± 2.28 b	8.4 ± 1.85 b	31.8 ± 5.41 b	22.6 ± 2.8 b	79.2 ± 16.44 b
20	100 a	4.8 ± 0.74 b	19.6 ± 1.85 b	11.8 ± 2.31 a	36.2 ± 2.48 ab	24.6 ± 2.41 b	42 ± 8.06 c
30	50 ± 31.6 b	8.6 ± 0.8 a	4 ± 0.63 c	12.8 ± 0.97 c	25.6 ± 2.05 c	17.2 ± 1.16 c	15.4 ± 1.8 d
Control (unstored)	100a	3.4 ± 1.02 c	33.8 ± 5.03 a	2.8 ± 0.74 c	40 ± 4.24 a	31 ± 7.12 a	255 ± 50.4 a
LSD	20.859	1.119	4.018	2.094	5.071	5.42	17.053

Means followed by the same small letter in a column are not significantly differences at the 5% level of probability (Duncan's Multiple Range Test)

Table (8). Effect of different storage periods on the biological characters of *R. cardinalis* adult stored at 10Cº

Storage Periods (days)	Survival of adult from storage period	Duration in days ±SE					
		Pre-oviposition	Oviposition	Post. ovi.position	Female longevity	Male longevity	Fecundity/ female
5	100 a	3 ± 0.63 a	21.6 ± 3.13 b	3 ± 0.63 c	27.4 ± 4.96 b	12.6 ± 0.24b	92.8 ± 7.8 b
10	100 a	3 ± 0.63 a	20.4 ± 7.2 b	3.8 ± 1.32 c	27.4 ± 7.19 b	18 ± 2.89 bc	52.4 ± 17.5 c
20	80 ± 10 b	5.2 ± 3.48 a	19 ± 11.36 b	15.4 ± 7.28 b	39.6 ± 9.85 a	14.8 ± 3.8 c	74.2 ± 49.24 bc
30	80 ± 18 b	4 ± 0.63 a	13.6 ± 2.05 b	28.8 ± 2.13 a	46.4 ± 3.72 a	35.4 ± 5.71 a	11.2 ± 2.31 d
Control (unstored)	100 a	3.4 ± 1.02 a	33.8 ± 5.03 a	2.8 ± 0.74 c	40 ± 4.24 a	31 ± 7.12 a	255 ± 50.4 a
LSD	13.99	2.51	9.79	5.12	9.45	6.89	36.48

Means followed by the same small letter in a column are not significantly differences at the 5% level of probability (Duncan's Multiple Range Test)

REFERENCES

Abdel-Salam, A. H. (2001). Cold storage of predaceous insects for using in biological control programs. Proceeding, Integrated pest management, 1st Cong., Cairo University, 22-23 April 183-190.

Abdel-Salam, A. H. and Abdel-Baky, N. F. (2000). Possible storage of *Coccinella undecimpunctata* (Col., Coccinellidae) under low temperature and its effect on some biological characteristics. J. Appl. Entomol., 123 : 169-176.

Ayvaz, A., E. Karasa, S. Karaborklu, and A. Tuncbilek. (2008). Effects of cold storage, rearing temperature, parasitoid age and irradiation on the performance of Trichogramma evanescens Westwood (Hymenoptera: Trichogrammatidae). J. Stored Prod. Res. 44: 232–240

Bigler, F., (1994). Quality control in Trichogramma production. In:Wajnberg, E., Hassan, S.A. (Eds.), Biological Control with Egg Parasitoids. CAB International, Wallingford, UK, pp. 93–111.

Caltagirone, L.E and Doutt, R.L., (1989). The history of the vedalia beetle importation to California and its impact on the development of biological control. Annu. Rev. Entomol. 34, 1–16.

Costat, Software (1990). Microcomputer program analysis Version 4.2, CoHort Sofware, Berkeley, CA

Coudron, M.R. Ellersieck and K.S. Shelby (2007). Influence of diet on long-term cold storage of the predator *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae) Biological Control, In Press,Corrected Proof, Available online 29 April 2007

Doutt, R.L., 1964. The historical development of biological control. In: DeBach, P. (Ed.), Biological Control of Insect Pests and Weeds.Chapman and Hall, London, p. 844.

Gagne I. and D. I. Coderre (2001) . Cold storage of *Coleomegilla maculata* larvae. Biocont. Sci. Technol. 11(3): 361-369.

Greenberg, S.M., Nordlund, D.A., King, E.G., 1996. Mass production of Trichogramma spp.: experiences in the former Soviet Union,China, the United States and western Europe. Biocontrol News Inform. 17 (3), 51N–60N.

Kui Liu, Buli Fu, Jiangrong Lin, Yueguan Fu, Zhengqiang Peng, and Qi'an Jin (2014). Effect of Temperatures and Cold Storage on Performance of *Tetrastichus Brontispae* (Hymenoptera: Eulophidae), a Parasitoid of *Brontispa longissima* (Coleptera: Chrysomelidae). J. Insect Sci. 14(257):

Leopold, R.A., 1998. Cold storage of insects for integrated pest management. In: Hallman, G.J., Denlinger, D.L. (Eds.), Temperature Sensitivity in Insects and Application in Integrated Pest Management. Westview Press, Boulder, CO, pp. 235–267.

Miller, J. C. (1995). A comparison of techniques for laboratory propagation of a South American ladybeetle, *Eriopis connexa* (Coleoptera: Coccinellidae). Biol. Control. 5 (3): 462-465.

Montgomery, M. E., H. Wang, D. Yao, W. Lu, N. Havill, and Guangwu, Li. (2002). Biology of *Scymnus ningshanensis* (Coleoptera: Coccinellidae) a Predator of Adelges tsugae (Homoptera: Adelgidae), pp. 181 –188. In Proceedings, The Hemlock Woolly Adelgid Symposium, February 2002, East Brunswick, NJ. Agric. Exper. Sta. Rutgers Univ., New Brunswick, NJ

Pitcher, S. A., M. P. Hoffmann, J. Gardner, M. G. Wright, and T. P. Kuhar. (2002).Cold storage of *Trichogramma ostriniaereared* on *Sitotroga cerealella* eggs. Biocontrol 47: 525–535.

Quezada, J.R., DeBach, P., 1973. Bioecological and population studies of the cottony-cushion scale, *Icerya purchasi* Mask., and its natural enemies, *Rodolia cardinalis* Mul. and *Cryptochaetum iceryae* Will.,in Southern California. Hilgardia 41, 631– 688.

Ravensberg, W.J., 1992. Production and utilization of natural enemies in western European glasshouse crops. In: Anderson, T.E., Leppla, N.C. (Eds.), Advances in Insect Rearing for Research and Pest Management. Westview, Boulder, CO, pp. 465–487.

Rawat-US.; SK. Sangal and AD. Pawar (1992). Development of *Chilocorus bijugus* Mulsant, a predator of San Jose scale, *Quadraspidiotus perniciosus* (Comstock) at different levels of temperature and relative humidity. J. Insect Sci. 5 (2): 137-140.

Umberto Bernardo, Luigi Iodice, Raffaele Sasso and P. Pedata. (2008). Effects of cold storage on *Thripobius javae* (=T. semiluteus) (Hymenoptera: Eulophidae). *Biocontrol Science and Technology* 18, 921-933.

Yigit A.; R. Canhilal and K. Zaman (1994). Cold storage of some natural enemies of citrus mealybug, *Planococcus citri* (Risso) (Homoptera: Pseudococcidae). Turkiye III. Biyolojik Mucadele Kongresi Bildirileeri, 25-28 Ocak 1994, Ege Universities Ziraat Fakultesi, Bitki Koruma Bolumu, Izmir. 137-146.

تـأثير التخـزين بالتبريـد علـى حيويـة المفتـرس الروداليـا كاردنـاليس *Rodolia cardinalis*

نجــدي فـــاروق عبـــد البـــاقي[1] ، محمـــد الســـيد رجـــب [1]، عبـــد البـــديع غـــانم [1]، محمود السيد النجار [2] و مصطفى مهران المتولي[2]
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يعتبر مفترس الفيداليا العامل الحيوي الأساسي للمكافحة الحيوية كمفترس للبق الدقيقي فى العالم ، لذلك تم دراسة تأثير التخزين بالتبريد على حيوية الأطوار المختلفة للمفترس. تم تخزين بيض المفترس لمدة 5، 10، 15 ، 20 : يوم على ثلاث درجات جراره 6 ، 10 ، 14 °م بينما الأعمار اليرقيه والطور الكامل تم تخزينهما لمدة 5، 10، 20، 30 يوم على درجتي حرارة 6، 10 °م. تخزين عذارى المفترس لمدة 5، 10، 20، 30 ، 40 يوم على ثلاث درجات جراره 6 ، 10 ، 14 °م. انخفضت نسبة الفقس بزيادة مدة التخزين عند تخزين بيض المفترس على درجة حرارة 6°م. بلغت نسبة الفقس للبيض المخزن على درجة 6 °م 84 ± 10.2، 14.00 ± 10.2 % للبيض عمر يوم و 78 ± 11. 6 ، 44.00 ± 12.6 % للبيض عمر يومان وذلك عند التخزين لمدة 5، 20 يوم على التوالى بينما البيض المخزن على درجة 10 °م بلغت نسبة الفقس 86 ± 4.8 ، 90 ± 0.6 % للبيض عمر يوم و 96 ± 4.8 ،، 92 ± 7.4 % للبيض عمر يومان وذلك عند التخزين لمدة 5، 20 يوم على التوالي. وتبين النتائج إمكانية تخزين بيض المفترس لمدة 20 يوم على درجة حرارة 10 °م. نسبة الحياه للعمر اليرقي الثالث والرابع أعلى من العمرين الأول والثاني عند التخزين على درجتي حرارة 6، 10 °م و أن العمر اليرقي الرابع كان أكثر تحملا لدرجات الحراره المنخفضة.

وقد تلخصت نتائج تلك الدراسه إلى إمكانية تخزين بيض هذا المفترس والعمر اليرقي الرابع لمدة 20 يوم على درجة حرارة 10 °م والعذارى والطور الكامل لمدة 20 يوم على درجة حرارة 6 °م دون التأثير على حيوية الأطوار المختلفه للمفترس و أن التخزين بالتبريد يمكن أن يستخدم فى تخزين المفترس لحين الحاجه إليه وإطلاقه فى برامج المكافحه كعنصر من عناصر الزراعة النظيفة.

Table (4): Effect of different storage periods on pupal stage and biological characters of *R. cardinalis* adult emerged from pupae stored at 6Cº.

Storage Periods (days)	Adult emergence (%)	Duration in days ±SE						Fecundity/ female
		Pupal stage after treatment	Pre-oviposition	Oviposition	Post-oviposition	Female longevity	Male longevity	
5	100 a	5.4 ± 0.48 b	3.6 ± 0.48 c	31.6± 10.05 a	3.8 ± 0.97 a	39 ± 9.18 a	18.4 ± 4.12 b	127.4 ± 56.19 b
10	90 ± 12.24 a	4.8 ± 0.4 c	4 ± 0.0 c	26 ± 8.5 a	3 ± 0.89 b	33 ± 8.92 a	18.2 ± 1.6 b	96.8 ± 25.24 b
20	80 ± 18.7 a	6.6 ± 0.48 ab	4 ± 0.63 c	30 ± 2.82 a	2.6 ± 1.49 b	36.2 ± 4.01 a	17.8 ± 5.34 b	81.8 ± 8.93 b
30	45 ± 18.7 b	5.8 ± 0.74 bc	7.4 ± 1.62 b	26.2 ± 7.54 a	2 ± 1.26 b	35 ± 7.04 a	12 ± 5.5 b	20.4 ± 7.49 c
40	30 ±2.99 b	6 ± 2.00 bc	12 ± 1.00 a	0.0 b	0.0 c	12 ± 1.00 b	3.5 ± 0.70 c	0.0 C
Control (un stored)	100 a	7.6 ±0.48 a	3.4 ± 1.02 c	33.8 ± 5.03 a	2.8 ± 0.74 a	40 ± 4.24 a	31 ± 7.12 a	255 ± 50.4 a
LSD	22.687	1.272	1.348	9.676	1.478	9.661	6.791	47.931

Means followed by the same small letter in a column are not significantly differences at the 5% level of probability (Duncan's Multiple Range Test).

Table (5): Effect of different storage periods on pupal stage and biological characters of *R. cardinalis* adult emerged from pupae stored at 10Cº.

Storage Periods (days)	Adult emergence (%)	Duration in days ±SE						Fecundity/ female
		Pupal stage after treatment	Pre-oviposition	Oviposition	Post-oviposition	Female longivity	Male longevity	
5	95 ± 5 ab	5.00 ± 0.0 b	4.8 ± 0.4 ab	30.2 ± 7.33 a	2.8 ± 1.16 abc	38 ± 6.51 a	25.6 ± 7 a	114.4 ± 35.13 b
10	100 a	3.6 ± 1.01 b c	5.6 ± 7.85 a	19.8 ± 10.8 ab	6.4 ± 3.44 a	31.8 ± 7.9 a	24.8 ± 5.5 ab	78.6 ± 42.14 bc
20	90 ± 20 ab	2.4 ± 0.48 bc	5.8 ± 1.46 a	21.4 ± 10.3 ab	5.8 ± 3.31 ab	32.8 ± 12.8 a	27.6 ± 10.2 a	60.2 ± 22.8 c
30	85 ± 10 b	0.8 ± 0.4 c	5.8 ± 7.6 a	10.4 ± 3.38 b	2 ± 1.26 b	18.4 ± 3.2 b	15.6 ± 2.8 b	17.8 ± 2.3 d
Control (unstored)	100a	7.6 ±0.48 a	3.4 ± 1.02 b	33.8 ± 5.03 a	2.2 ± 0.4 c	40 ± 4.24 a	31 ± 7.12 a	255 ± 50.4 a
LSD	13.19	2.50	1.78	12.19	3.32	11.38	10.305	40.65

Means followed by the same small letter in a column are not significantly differences at the 5% level of probability (Duncan’s Multiple Range Test).