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Influence of long-term cold storage on the quality of predatory mite, *Typhlodromus pyri* scheuten (Acari: Phytoseiidae)

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Abstract
Typhlodromus pyri is a commercially available predatory mite used to control broad pests in agricultural production. The inconsistent quality of mass-produced biocontrol agents is a major concern in augmentative biological control, where a few studies have tested. The ability to store predator eggs of *Typhlodromus pyri* at different low temperatures; 2, 5 and 10°C till five weeks was studied to investigate the effect of low temperature on some biological aspects of the predator for a long time. The storage at 10°C has shown the best results in upgrading some biological aspects, the consumption, the longevity and fecundity of predatory females compared with the control, where the longevity was 21.5 days and the fecundity was 40.6 eggs at 30°C. Also, there were significant differences when biological aspects are concerned. Increasing storage temperature and decreasing time of storage gave high fecundity rate near similar to the control treatment in addition to fertility of eggs. The cold storage of the predatory mite, *Typhlodromus pyri* eggs at 2°C caused reduction in female hatchability % and fecundity so, it can be recommended that, this type of storage should be avoided except when there are a high number of predatory eggs that can be stored for period up to 2 weeks till 4 weeks.

Keywords: cold storage, biological characteristics, predatory mite, *Typhlodromus pyri*

Introduction
Predaceous mites play an important role in the biological control of associated pests, such as phytophagous mites, scale insects and whiteflies. The most important natural enemies of tetranychid mites are phytoseiid mites, that have a well-known capacity to suppress pest mite populations in diverse cropping system (McMurtry and Croft, 1997; McMurtry *et al.*, 2013; Easterbrook *et al.*, 2001; Colfer *et al.*, 2004) [11, 12, 5, 2]. Members of the family Phytoseiidae proved to have a high predaceous efficiency, worldwide distribution and a large number of species which exceeds 1700 species (Walter, 1992) [15]. The predatory mite of genus, *Typhlodromus* attacks mites, including the two-spotted spider mite. *Typhlodromus* is well adapted to hot and dry conditions. *Typhlodromus pyri* is considered one of the species belonging to family Phytoseiidae. Storage of mass-reared phytoseiid mites is an important issue for commercial suppliers. Commercial producers often are required to produce large numbers of phytoseiid mites during specific times of the year. This may not be easy to accomplish, so commercial suppliers are interested in the possibility of stockpiling predatory mites for shipment during peak times. Storage for more than one month is considered long-term storage (Leopold, 1998; Van Lenteren and Tommasini, 1999) [8, 14]. *Amblyseius cucumeris* (Oudemans) larvae or adults can be stored at 8–9°C for 10 weeks and *Phytoseiulus persimilis* Athias-Henriot larvae or adults can be stored at 7.5°C for 4 weeks (Leopold, 1998) [8]. Although chilling causes high rates of mortality during storage (Luczynski *et al*., 2008) [9]. The availability of food also affects the survival of predatory mites (Morewood, 1992; Bogdanov *et al*., 2008; Gotoh and Tsuchiya, 2009) [10, 1, 6]. The present study aims at determining the capability to store the predator eggs for a long time under low temperature, when needed to transport the stored eggs to another place that required long periods in transportation that can be traded commercially.

Materials and Methods
Rearing of the predatory mite, *Typhlodromus pyri*
A stock culture of the predatory mite; *Typhlodromus pyri* was collected from the Soybean crop at Sharkia Governorate, Egypt. Leaf discs, 3 cm diameter of mulberry (*Morus alba* L.) were used as a substrate for rearing the predator and put in Petri-dishes on a piece of cotton wool, each disc was lined with a wet cotton barrier. Drops of water were added daily to maintain suitable moisture for the predator. Whenever, leaf substrate began to deteriorate, it was changed by fresh one and enough numbers of *Tetranychus urticae* at different stages were offered as prey. The experiments were carried out under constant conditions at 28±2ºC and 65±5% R.H.

Bioassay test
Three groups of low temperatures; 2, 5 and 10ºC for cold storage were divided into five treatments; one, two, three, four and five weeks of storage, including three replicates (cups) for each treatment. The cup contained 10 newly deposited eggs of *Typhlodromus pyri,* placed on mulberry leaf discs in a covered cylindrical plastic cup (2.8 cm diameter and 2 cm deep) with filter paper on its bottom. The humidity was maintained by adding little water drops on the filter paper when needed. Three cups were kept at 30ºC without storage as a control, then every week 3, cups withdrawn from each low temperature for five weeks and each cup contained 10 eggs that transferred to the incubator at 30±1ºC. Egg incubation period, hatchability % and life cycle were recorded. Also, the cups with newly hatched larva reared until adulthood. Before the final molt of the

female, was confined singly and one adult male was introduced into the replicate for mating that supplied with known numbers of *Tetranychus urticae* immatures when needed as a prey. After one day, the male was removed then longevity, consumption and fecundity were calculated.

Statistical analysis

Data were subjected to statistical analysis using one-way analysis of variance, ANOVA (Duncan, 1955) [4].

Results

The results in table (1) show the effect of low temperatures on *Typhlodromus pyri* eggs incubation and hatchability % after 1 to 5 weeks. There was an inverse relation between the storage temperature and the incubation period of *Typhlodromus pyri* eggs, when the storage temperature increased the incubation period decreased, that reached 7.7 days at 2ºC. While, the incubation period decreased until reached 3.9 days at 10ºC after the first week of storage. Likewise, the storage from the beginning of the second week to the fifth was found to become 15 days at 2ºC and 8.5 days at 10ºC. In case of the hatchability %, it was found to increase gradually with the cold storage at 2-10ºC during the storage weeks. Hatchability % was 41.4-95.8% after the first week of storage, while it ranged from 9.8-68.7% after the fifth week of storage at the same temperatures.

This study concluded that, the storage temperature of 10ºC was the best for the eggs storage, where the incubation period of eggs was decreased in addition to preserving the fertility of the eggs during the five weeks of storage.

Table 1: Effect of low temperature on incubation period and hatchability % of *Typhlodromus pyri* after five weeks of storage after incubation at 30±1°C

Cold storage time (weeks)	Cold storage temperature (°C)					
	2		5		10	
	Incubation period (days)	Hatchability %	Incubation period (days)	Hatchability %	Incubation period (days)	Hatchability %
1	7.7±0.11c	41.4±3.50b	5.2±0.07c	77.1±2.57b	3.9±0.07c	95.8±3.06a
2	9.4±0.67b	28.6±1.09c	7.1±0.45b	60.0±1.09c	4.8±0.23c	89.3±3.02a
3	13.1±0.58a	14.3±0.74d	9.7±0.24b	40.0±2.03d	6.8±0.44b	75.4±3.98b
4	14.1±0.67a	12.8±0.88d	11.2±0.53a	17.9±0.37e	6.9±0.34b	71.3±3.43b
5	15.0±0.89a	9.8±0.86e	12.6±0.63a	10.1±0.98e	8.5±0.53a	68.7±2.00c
Control	2.5±0.12d	97.1±3.56a	2.5±0.12d	97.1±3.56a	2.5±0.12c	97.1±3.56a

Means in columns followed by the same letter are not significantly different at $p \leq 5\%$ (Duncan's multiple range test, 1955). ± Standard Error
Control: Incubation at 30±1°C without storage

The data in table (2) show that, when the eggs of *T. Typhlodromus pyri* were stored at low temperatures of 2, 5 and 10ºC, the time to reach the adult stage was reduced and the life cycle of individuals resulting from hatching eggs stored at previous temperatures was also reduced. It was found that the life cycle decreased as the storage temperature increased, as it ranged between 15.9-11.3 days, after the first week of storage, while the life cycle increased until it reached 24.1-21.9 days after the fifth week of storage. The adult individuals lived different periods of longevity due to different storage temperatures and storage times. It was found that cold storage temperatures of 2, 5 and 10ºC cause the individuals to live for a short period after the fifth week of storage, but the maximum longevity for the individuals reached 17.2 days at 10ºC after a week of storage.

It was important and necessary to study the predatory behavior of *Typhlodromus pyri*, as it is one of the most important types of predatory mites used in the field of biological control after cold storage of the eggs, in addition to the ability of females to reproduce by laying eggs.

Table 2: Effect of low temperature on life cycle and longevity of *Typhlodromus pyri* after five weeks of storage after incubation at 30±1°C

Cold storage time (weeks)	Cold storage temperature (°C)					
	2		5		10	
	Life cycle (days)	Longevity (days)	Life cycle (days)	Longevity (days)	Life cycle (days)	Longevity (days)
1	15.9±1.10b	12.5±0.22b	13.0±0.81b	14.4±0.92b	11.3±0.78c	18.2±0.77a
2	19.0±1.07b	10.2±0.6b	15.2±1.34b	12.5±0.12b	12.9±1.70c	16.5±0.32a
3	21.4±1.76a	7.3±0.52c	19.9±1.08a	9.2±0.88c	16.1±1.13b	14.3±1.00b
4	22.4±1.89a	5.4±0.35c	21.8±1.61a	6.2±0.74c	15.7±1.90b	13.9±0.96b
5	24.1±1.03a	3.3±0.21d	22.4±1.25a	4.0±0.30d	21.9±1.01a	9.2±0.44c
Control	9.7±0.73c	21.5±0.98a	9.7±0.73c	21.5±0.98a	9.7±0.73c	21.5±0.98a

Means in columns followed by the same letter are not significantly different at $p \leq 5\%$ (Duncan's multiple range test, 1955).± Standard Error Control: Incubation at 30±1°C without storage

The results in table (3) and figure (1) indicate that the consumption increased as the storage temperature increased and the storage time decreased. So, it was found that the maximum consumption of the female reached 70.8 individuals at 10ºC after the first week, but by increasing the storage period for the same temperature, the consumption decreased until it reached 27.2 individuals after the fifth week. Special results were obtained for the biology of the predator, *Typhlodromus pyri*, starting from the incubation period of eggs and the percentage of hatching down to the surviving of individuals, but the fecundity of individuals resulting from this cold storage is the most important biological characteristic to judge the success of cold storage. The results in table (3) and figure (2) reveal that the total amount of eggs laid for the female was 36.6 eggs at 10ºC after a week of storage. This percentage remained decreased as the weeks of storage progressed, until it reached 15.9 eggs after the fifth week, and gave the fecundity of the females, which did not store cold storage and were placed at 30ºC, their fecundity was 40.6 eggs. In view of the remaining storage temperatures; 2 and 5ºC, the temperature of 5ºC gave almost satisfactory results compared to the lowest degree (2ºC), where the females were unable to lay eggs.

Table 3: Effect of low temperature on fecundity and consumption of *Typhlodromus pyri* after five weeks of storage after incubation at 30±1°C

Cold storage time (weeks)	Cold storage temperature (°C)					
	2		5		10	
	Consumption	Fecundity	Consumption	Fecundity	Consumption	Fecundity
1	46.7±2.25^{b}	25.1±1.66^{b}	54.6±3.69^{b}	31.4±1.44^{b}	70.8±4.87^{b}	36.6±1.79^{a}
2	48.5±2.69^{b}	15.2±0.78^{c}	56.0±3.49^{b}	23.5±1.28^{c}	76.4±4.79^{a}	32.0±1.08^{b}
3	40.2±2.17^{c}	10.1±0.82^{d}	50.3±2.98^{c}	20.8±0.94^{c}	65.3±3.69^{b}	28.2±1.45^{b}
4	37.3±2.01^{c}	10.0±0.58^{d}	47.3±2.84^{c}	18.5±0.89^{c}	62.3±3.29^{b}	27.5±1.87^{b}
5	10.6±0.82^{d}	0.0±0.00^{e}	11.1±0.78^{d}	10.0±0.52^{d}	27.2±1.23^{d}	15.9±0.24^{c}
Control	82.7±3.76^{a}	40.6±2.07^{a}	82.7±3.76 ^{a}	40.6±2.07^{a}	82.7±3.76^{a}	40.6±2.07^{a}

Means in columns followed by the same letter are not significantly different at $p \leq 5\%$ (Duncan's multiple range test, 1955). ± Standard Error
Control: Incubation at 30±1°C without storage

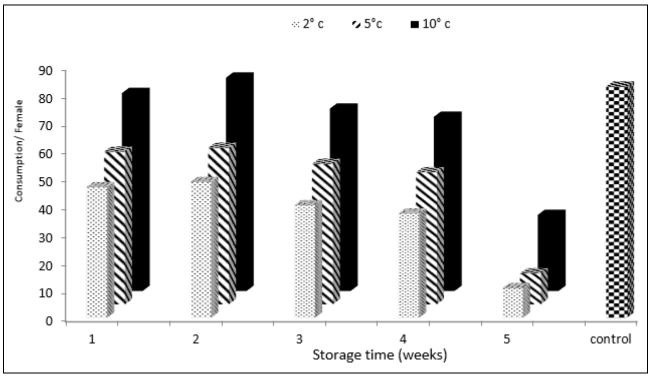

Fig 1: Effect of cold storage of *Typhlodromus pyri* eggs for five weeks on consumption at 30±1°C

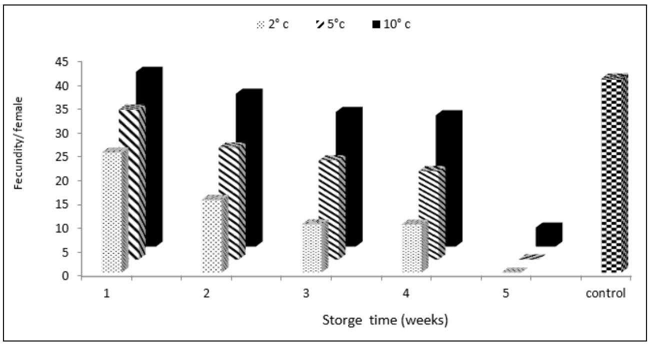

Fig 2: Effect of cold storage of *Typhlodromus pyri* eggs for five weeks on fecundity at 30±1°C

Discussion

Climatic conditions play an important role in determining the biology of phytoseiidae mite, *Typhlodromus pyri*. During laboratory mass rearing, cold storage can be practiced when prey is scarce or under other food deficiency. In addition, temperature usually drops to 10ºC or less during some winter nights in many countries. Thus, for these two reasons the experiment was conducted to investigate the effect of low temperatures of 2, 5 and 10ºC on some biological characteristics of *Typhlodromus pyri*. Cold-storage time had an important effect on the survival of *Typhlodromus pyri* females. In this study, 1 and 5 weeks of cold storage at 5 or 10 °C did not appear to have a significant effect on the biological characteristics for *T. pyri* females, but storage for 5 weeks was harmful at 2 °C. This study concluded that, the storage temperature of 10ºC was the best for the *T. pyri* eggs storage, where the incubation period of *T. pyri* eggs was decreased in addition to preserving the fertility of the eggs during the five weeks of storage. Increasing storage temperature and decreasing time of storage gave high fecundity rate near similar to the control treatment in addition to fertility of eggs. The obtained results are nearly similar to the previous studies that indicate of Dhillon and Sharma (2007) [3] reported that eggs of *Helicoverpa armigera* can be stored for 10 days at 10°C with a hatchability of >75%. Zaher *et al*. (2007) [16] reported that, eggs of phytoseiidae predacious mites; *Phytoseuilus persimilis* can be stored for a month at a low temperature of 9ºC or less. Also, Morewood (1992) [10] reared adult *Phytoseuilus persimilis* successfully at 5 and 8ºC for 4 weeks and survival rate was 97% after 4 weeks and 80% after 6 weeks at 7.5ºC, *P. persimilis* females, within a few days after their return to room temperature, laid eggs at the same rate as females taken directly from rearing cultures. Sinclair *et al*. (2003) [13] showed the cooling influence on the insect's physiological responses of *Helicoverpa armigera*. Hamamura *et al*. (1978) [7] found that survival of *P. persimilis* held at 5, 7.5, or 10 °C at 95% RH after 12 days was 72, 76, or 84%, respectively. But after 25 days, the survival rates were 20, 28, or 12%, respectively at 5, 7.5, or 10 °C.

Conclusion

Generally, eggs of *Typhlodromus pyri* tolerate low temperature than its females. Accordingly, when food is scare, the eggs can be stored for a month at low temperatures. This study indicated that *Typhlodromus pyri* reveals remarkable tolerance to cold storage for 4 weeks, where the predator manages to consume, survive and deposit eggs successfully under low temperatures. This study manages to store the predator eggs for a long time under low temperatures when needed to be used in biological control of mite pests or to transport it to another place requiring a long period of time in commercial trading. So that it can be recommended, particularly under obligated case when there are a high number of predatory eggs.

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