Influences of Cold Storage Period and Rearing Temperature on the Biological Traits of *Trichogramma oleae*

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ABSTRACT

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Egg parasitoid *Trichogramma oleae* could be useful in biological control programs of olive pests. The aim of the present study is to evaluate the effects of a series of cold storage periods (from 1 to 10 weeks at 4 ± 1°C, RH = 75 ± 10% and full darkness), and rearing temperatures on the traits (emergence rate, fecundity, longevity and walking speed) of Tunisian strain egg parasitoid *T. oleae*. It seems that *T. oleae* pupae are amenable to cold storage for three weeks without affecting emergence rate, longevity and walking speed of adults. The parasitization performance of F1 progeny from pupae stored up to 3 weeks was not affected. All these performances were greatly affected after longer storage periods. The elevation of rearing temperature from 15 to 35°C led to a significant decrease in the developmental period of *T. oleae* pupae, and reduced longevity and fecundity of wasps. Adult longevity and emergence rate were significantly affected by temperature. In fact, the mean longevity of adults was 18.22 days at 15°C and decreased to 4.58 days at 35°C, whereas the emergence rate was 87.56% at 15°C but significantly decreased to 45.32% at 35°C. At high temperatures, *T. oleae* remains active and it can be a good candidate for biological control of Lepidoptera species in warm climates (temperatures near to 30°C).

Keywords: Cold storage, egg parasitoid, *Ephestia kuehniella*, mass rearing, *Trichogramma oleae*

In several parts of the world, *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) are the most widely used natural enemies, partly because they attack many important insect crop pests and they are also easy to mass rear.

The successful use of the field release of mass-reared *Trichogramma* species has reduced the applied pesticide levels (14, 15, 16, 22, 31). They attack the life cycle of the phytophagous and can attain high parasitism rates (12, 25). The achievement of this biological control relies not only on the time and the amount of natural enemies released, but also on their quality (emergence, longevity, fecundity, and searching capacity) (1, 8, 10, 34).

Cold storage technique must ensure the availability of sufficient numbers (33) and quality of egg parasitoids (1) 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

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use in research (11, 21, 30). Besides, cold storage can permit a more cost-effective production schedule (9) providing a means to conserve biological control agents when not immediately needed (29).

A number of *Trichogramma* species have been tested by exposing the target insects to cold storage conditions (3, 17, 20, 26, 36). It is important to test the amenability of each *Trichogramma* species to cold since not all of them are able to be cold-stored (33).

Temperature is one of the important factors affecting the distribution and abundance of egg parasitoids (23). All demographic parameters of *Trichogramma* spp. (developmental rate, fecundity, and longevity) are affected by temperature (4). Knowledge of thermal requirements and the effect of temperature must be evaluated before mass rearing and release of the egg parasitoids (23).

The purposes of the present work are to evaluate the effect of a range of cold storage periods on the subsequent performance of Tunisian strain of *T. oleae*, to assess the effects of such storage conditions on the quality of the F1 progeny and finally, to show the effects of different rearing temperatures on this strain quality.

MATERIALS AND METHODS

Insect rearing.

The Tunisian strain of *T. oleae* was obtained from parasitized eggs of *Prays oleae* originating from an olive orchard (Sfax, Tunisia) in September 2006. This egg parasitoid have been reared in the laboratory, on ultraviolet-irradiated eggs of *Ephestia kuehniella* at 25 ± 1°C, 75 ± 5% RH with a photoperiod of 14 h:10 h (L:D).

It is noted that *T. oleae* is completely thelytokous (28) and is mostly related to *T. evanescens* (18, 37).

Influence of cold storage.

Fifty (±10) freshly collected eggs of *E. kuehniella* were glued onto pieces of cardboard with a water solution of non-toxic glue. The cards were placed separately in glass tubes (15 cm × 2.5 cm) for 24 h with five females of *T. oleae* 1 day-old each at 25 ± 1°C, 75 ± 5% RH with a photoperiod of 14 h:10 h (L:D). After 7 days of incubation, the parasitoid pupae were stored as blackened host eggs at 4°C, 75 ± 5% RH and in full darkness for up to 10 weeks. Similar temperatures (around 4°C) are used for cold storage for other *Trichogramma* spp. (*T. chilonis*, *T. evanescens*, *T. pintoi*, *T. cordubensis*…) (11, 17, 35). The pupal stage was used because it showed better tolerance to cold storage compared to other embryonic stages in several *Trichogramma* spp. (17).

A control was kept at standard rearing conditions. Each week, once the storage period was over, the parasitized eggs in the glass tubes were transferred from storage to the bioclimatic chamber and maintained at standard rearing conditions to monitor emergence of adult parasitoids. Twenty replicates were weekly tested at each storage period.

The effect of treatments (different periods of cold storage) on the quality of the stored pupae was evaluated by measuring the following parameters: percentages of adult emergence determined for the parental (P) and F1 generations, longevity and walking speeds.

To evaluate longevity, 24 hold females were used for each treatment. The females were transferred to individual glass tubes with a honey drop as food (replaced every 48 h) and placed at 25 ± 1°C, 75 ± 5% RH with a photoperiod of 14 h:10 h (L:D). Mortality of the wasps was recorded daily.

For calculating walking speed, the walking path of each wasp was traced on millimeteric paper used as background. The number of 1 mm traveled during 10 s of continuous walking was counted and walking speed was calculated (32).Ten females were used to calculate the mean of walking speed.

Influence of rearing temperature.

Twenty females of *T. oleae* aged more than 24 h were used to measure fecundity during three days at each temperature. Each female was placed in a glass tube with a card containing 100-120 sterilized eggs of *E. kuehniella* and a honey drop as a food source. The glass tubes were storage at different temperatures (15, 20, 25, 30, and 35°C). Egg cards were changed every day. After three days, the wasps were removed, the number of parasitized eggs for the first 3 days and the percentage of adult emergence were determined and recorded.

Newly emerged female were isolated in a glass tube. Longevity was evaluated by noting daily the number of dead females; 20 females were used at each temperature. A honey drop was added until the wasp died. The wasps drowning in honey droplets were discounted. The mean developmental time of parasitoids from egg to pupae (blackened egg) and from egg to adult was determined at the five different temperatures (15, 20, 25, 30, and 35°C).

Statistical analysis.

Data from the experiments were analyzed with ANOVA using SPSS version 18. Percentage data was transformed using arcsine $\sqrt{x}$ before ANOVA. Means were separated at the 5% significance level by using least significant difference (LSD) tests.

RESULTS

Influence of cold storage.

Developmental time from pupa to adult emergence was approximately 7 days in the control treatment and varied around 8 to 9 days for all the samples in each treatment.

Due to low adult emergence after 9 and 10 weeks of cold storage, emergence, longevity and the walking speed were not assessed for these treatments.

Stockpiled at 4°C, *T. oleae* pupae allowed a great number of adult emergence during all three first weeks of storage periods; we distinguish no difference between treatments and the control group up to three weeks of trial. The proportion of emerged adults decreased significantly with increasing the period of cold storage (Fig. 1). A significant decrease was observed from the 4th week and over ($F_{8,171}$ = 59.691; P < 0.0001). In the control, 93.04% of adults emerged but this parameter decreased to 70.70%, 64.89%, 44.31%, 12.26%, and 6.32% after 4, 5, 6, 7, and 8 weeks of storage, respectively.

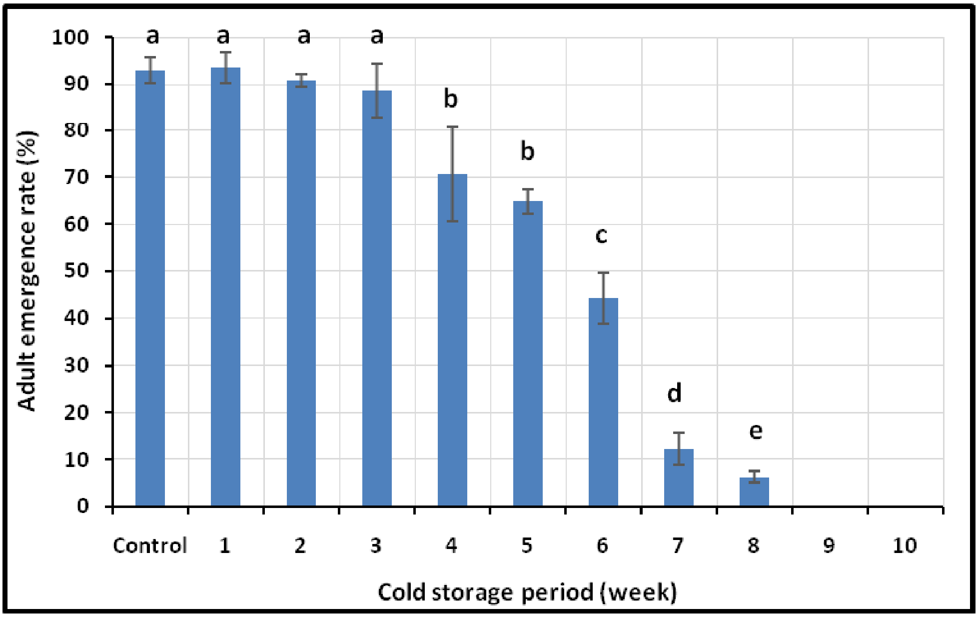

Fig. 1. Percentage of emerged adults of *Trichogramma oleae* (mean ± SE) as function of cold storage period. Bars sharing the same letter are not statistically different at $P < 0.05$ based on LSD tests.

With the increase of cold storage duration, longevity and walking speed of the adults from stored pupae were significantly decreased ($F_{8,130} = 99.828$; $P < 0.0001$ and $F_{7,72} = 35.553$; $P < 0.0001$, respectively). A significant linear regression between cold storage and longevity was observed (y = -1.312 x +11.072, R_2= 0.9369, t = 32.61; df = 1; $P < 0.0001$). The same trend was also noticeable for walking speed (y = -0.5334 x + 5.7012, R_2 = 0.9011, t = 23.64; df = 1; $P < 0.0001$) (Fig. 2).

The F1 progeny of adults from stored pupae were able to parasitize as well as the control up to 3 weeks of storage, but adults from longer storage periods significantly parasitize less ($F_{8,171} = 39.894$; $P < 0.0001$). Adult emergence of F1 generation was significantly altered ($F_{4,95} = 2.30$; $P < 0.085$) ranging from 33 to 98% (Table 1).

Influence of rearing temperature.

The results in Table 2 describe the effect of different temperature regimes on developmental period of Tunisian wasp *T. oleae*. Increasing temperature from 15 to 35°C resulted in a significant decrease in the total developmental period and the egg to pupa developmental period. The developmental period from egg to pupae was significantly longer at 15°C than at other temperatures ($F_{4,95} = 19.781$; $P < 0.0001$); this period was 9.56 days at 15°C and decreased to 2.38 days at 35°C. The same tendency was observed in the total developmental period from egg to adult ($F_{4,95} = 21.851$; $P < 0.0001$). It varied from 16.39 days at 15°C to 8.13 days at 35°C (Table 2).

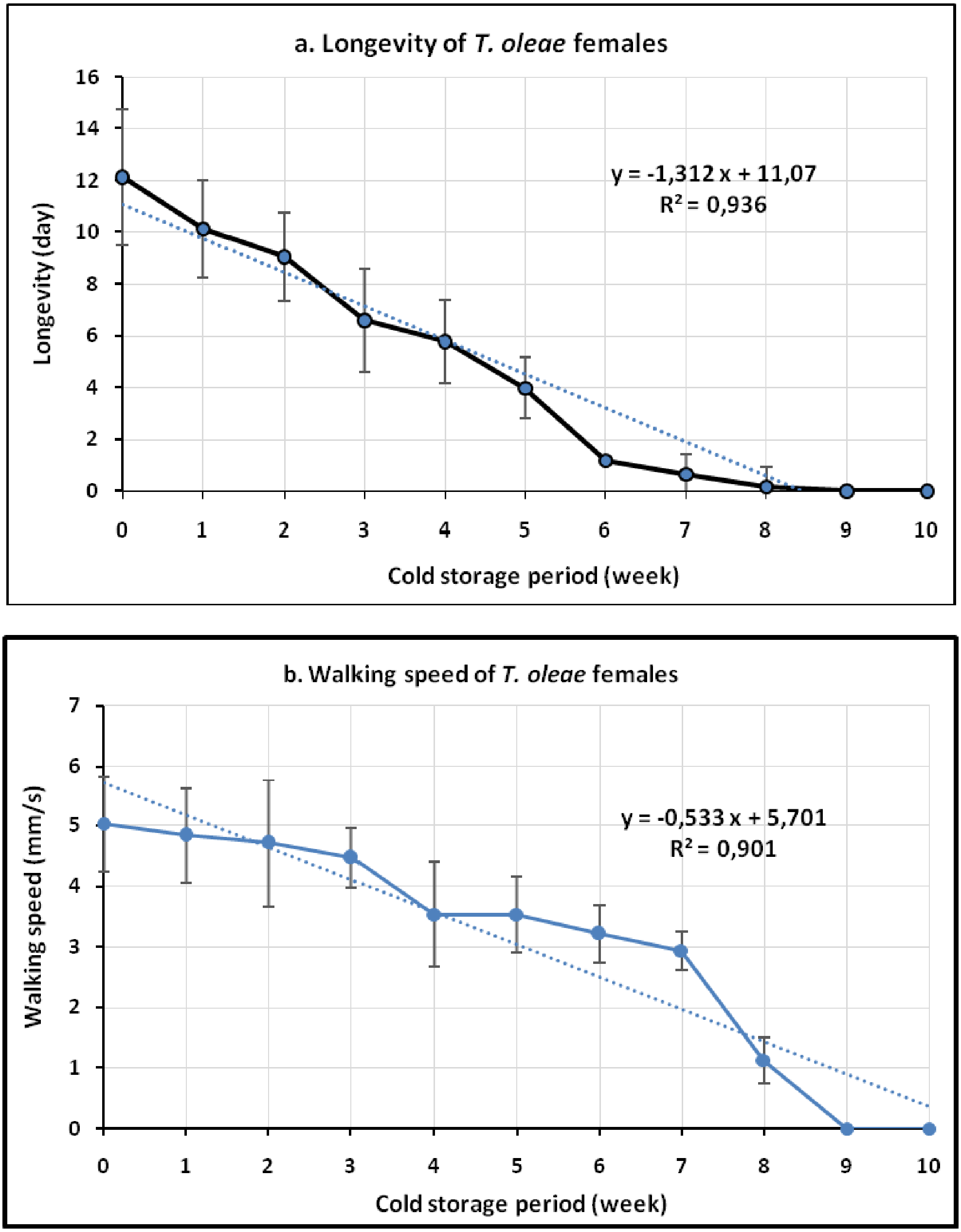

Fig. 2. Mean longevity (a) and walking speed (b) of cold stored *Trichogramma oleae* females depending on the incubation period tested.

Table 1. Effects of different cold storage periods at 4°C on F1 progeny of *Trichogramma oleae*

Storage (week)	F1 generation	
	Parasitization/female	Adult emergence rate (%)
Control	42.73 ± 7.14 a	98.21 ± 13.08 a
1	41.08 ± 2.36 a	90.67 ± 19.27 b
2	38.77 ± 4.39 a	95.36 ± 20.24 a
3	39.91 ± 3.64 a	93.69 ± 26.29 ab
4	21.09 ± 6.64 b	71.55 ± 17.86 c
5	15.90 ± 2.89 c	69.92 ± 12.48 c
6	8.73 ± 0.81 d	33.72 ± 10.35 d
7	2.73 ± 0.81 e	11.55 ± 8.95 e
8	0.00 f	0.00 f
9	0.00 f	0.00 f
10	0.00 f	0.00 f

[a] Means followed by the same letter in a column are not significantly different at $P < 0.05$ based on LSD tests.

Table 2. Influence of rearing temperature on the developmental period of *Trichogramma oleae*

Temperature (°C)	Developmental period (Day)	
	Egg to pupae	Egg to adult
15	9.56 ± 0.93 a	16.39 ± 0.73 a
20	6.35 ± 0.75 b	12.37 ± 0.37 b
25	4.21 ± 0.68 c	10.93 ± 0.51 c
30	2.88 ± 0.63 d	8.98 ± 0.71 d
35	2.38 ± 0.54 d	8.13 ± 0.63 d

[a] Means followed by the same letter in a column are not significantly different at $P < 0.05$ based on LSD tests.

The mean fecundity of females was not significantly different for the first 3 days at 25 and 30°C, but a pronounced decrease was observed at 35°C (for the first day, $F_{4,95} = 10.142$; $P < 0.0001$; for the second day, $F_{4,95} = 11.986$; $P < 0.0001$; for the third day, $F_{4,95} = 9.569$; $P < 0.0001$). The greatest fecundity was found on the first day of the oviposition period at all temperatures. The mean fecundity per female at the first day was 31.09 at 25°C but decreased to 2.21 at the third day for the females reared at 35°C (Table 3). The results show that most of the eggs are deposited during the first few days.

Adult longevity was significantly affected by temperature. Increasing temperature led to a decrease in longevity ($F_{4, 92} = 0.678$; $P < 0.0001$), whereas the mean longevity of adults was 18.22 days at 15°C; it decreased to 16.31, 12.20, 8.92, and 4.58 at 20, 25, 30, and 35°C, respectively (Table 3). The adult emergence rate was not significantly different at temperatures between 15 and 30°C, ranging from 84% to 89% ($F_{4,92} = 0.361$; $P = 0.897$), while at 35°C, the adult emergence rate decreased significantly ($F_{4,92} = 0.732$; $P < 0.0001$) (Table 3).

Table 3. Influence of rearing temperature on parasitization, adult emergence and longevity of *Trichogramma oleae*

Temperature (°C)	First 3 days parasitization/female			Adult emergence rate (%) of F1 progeny	Longevity of parental female (Day)
	1	2	3		
15	17.15 ± 3.68 a	8.35 ± 2.33 a	8.23 ± 1.98 a	87.56 ± 8.36 a	18.22 ± 3.51 a
20	25.33 ± 5.32 b	11.21 ± 3.02 b	9.20 ± 2.22 a	89.21 ± 6.73 a	16.31 ± 3.85 b
25	31.09 ± 3.59 c	17.21 ± 3.84 c	12.72 ± 3.41 b	84.95 ± 5.36 a	12.2 ± 2.15 c
30	30.87 ± 4.82 c	16.77 ± 3.14 c	12.60 ± 2.55 b	88.62 ± 7.21 a	8.92 ± 3.47 d
35	21.82 ± 5.07 d	5.82 ± 2.55 d	2.21 ± 1.08 c	45.32 ± 5.33 b	4.58 ± 1.24 e

[a] Means followed by the same letter in a column are not significantly different at $P < 0.05$ based on LSD tests.

DISCUSSION

Low temperature stopped the embryonic development of *T. oleae* pupae. If embryonic development occurred at 4°C, we would expect a shorter time until emergence in the treatments versus the control. This cease in the development during storage was described in other storage studies for other *Trichogramma* spp. at temperatures between 2 and 10°C (5, 17). Moreover, in our study, the time required for adult emergence for cold stored pupae ranged between 24 and 48 h later than the control group. This difference could be attributed to delays in returning back to normal metabolic conditions after being cold stored for a prolonged period (13, 33).

This study showed that *T. oleae* pupae are amenable to cold storage. The data provided by our experiments indicate that *T. oleae* stored for up to three weeks without much loss of performance. Despite some reduction in traits of female wasps like longevity and walking speed of adults from pupae stored for 3 weeks, the rate of parasitization was not significantly different from the control group. Similar results were reported for *T. ostriniae* stored at 6°C (29). However, prolonged storage at low temperatures had a detrimental effect on the biological traits of *T. oleae* (Fig. 1). This phenomenon was also recorded in other *Trichogramma* spp. (17, 35). One possible explanation suggested a reduction in fat body reserves during cold storage that affected greatly the performance of adults such as the mobility capacity (7).

The fecundity of the stored female wasps decreased with increasing the storage period beyond 3 weeks. Since the parasitization performance of F1 progeny from pupae stored for up to 3 weeks was not affected. The optimum cold storage periods have been reported for different *Trichogramma* species (17, 20, 26). In fact, fecundity and longevity of *T. achaea*, *T. chilonis* and *T. japonicum* declined after storage for 2 weeks at 2 and 5°C and after 3 weeks at 10°C (17). A significant decline in parasitoid emergence was also reported for *T. cacoeciae*, *T. brassicae* and *T. evanescens* after storage at 4°C for 3 weeks (26).

Rearing temperature affected longevity, fecundity, and developmental period of the parasitoid. The present experiments show that *T. oleae* longevity is inversely related to the temperature. Other studies have indicated a mean longevity of parasitoids near to 25 days for *T. minutum* (38) and 11 days for *T. platneri* (24) at 25°C. *T. oleae* has an intermediate longevity at 25°C about of 12.2 ± 2.15 days.

At high temperatures (35°C), according to some reports, longevities are around 8 days for unfed *T. minutum* (38) and 4 days for *T. brevicapillum* and *T. pretiosum* (27). Furthermore, *T. oleae* has a relatively short longevity at 35°C but should be a good candidate for biological control of Lepidoptera species at temperatures between 20 and 30°C.

The parasitization during first three days was not significantly different at 25 and 30°C, but decreased significantly at 35°C. With parasitism observed at 35°C, the number of wasps emerging from the blackened eggs was very low. Nevertheless, at 35°C, many parasitized eggs by *T. oleae* turned black and do not give adults. However, regardless the emergence of parasitoids, parasitized and blackened eggs were killed indicating that *T. oleae* could be a good candidate for release as a biological control agent under warm climates.

Reared on *E. kuehniella* eggs, the total development period (from egg to adult) for *T. oleae* was 13.39, 12.37, 10.93, 8.98, and 8.13 days at 15, 20, 25, 30 and 35°C, respectively. At different rearing temperatures, *T. oleae* like many other *Trichogramma* species laid the majority of its eggs over the first day (32).

Storage of *Trichogramma* pupae at low temperature could be useful for biological control strategies (inundative or inoculative releases). The effectiveness of *Trichogramma* as biological agents could be increased when used in combination with other control measures (19). Some studies already proved that combination releases of partially sterile codling moth and *Trichogramma* wasp, reduced damage of this pest in large field cages and in apple orchards (2, 6). This strategy is more effective than any technique used alone.

Regarding the storage techniques, mass rearing of native species (adapted to the local environmental conditions) is important for releasing these species. Therefore, the study of the possible effects of storage techniques of *Trichogramma* species under laboratory conditions allowed their producers to stockpile the parasitoids for future release in the field.

RESUME

Gharbi N. 2014. Influences de la durée du stockage au froid et de la température d'élevage sur les caractéristiques biologiques de *Trichogramma oleae*. Tunisian Journal of Plant Protection 9: 143-153.

Le parasitoïde des œufs *Trichogramma oleae* pourrait être utile dans les programmes de lutte biologique contre les ravageurs de l'olivier. Le but de la présente étude est d'évaluer les effets d'une série de périodes de stockage au froid (1 à 10 semaines à T = 4 ± 1°C, HR = 75 ± 10% et une obscurité totale) et de températures d'élevage sur les performances biologiques (rapidité d'émergence, fécondité, longévité et vitesse de marche) de la souche tunisienne du parasitoïde des œufs *T. oleae*. Il semble que les nymphes de *T. oleae* tolèrent le stockage au froid pendant trois semaines sans que le taux d'émergence, la longévité et la vitesse de marche des adultes ne soient modifiés. Le taux de parasitisme chez la descendance F1 issue des pupes stockées pendant trois semaines n'a pas également été affecté. Pour de longues périodes de stockage, toutes ces performances biologiques ont été significativement affectées. L'élévation de la température d'élevage de 15 à 35°C entraîne une diminution significative de la période de développement des nymphes de *T. oleae* et une réduction de la longévité et de la fécondité de ces guêpes. La longévité des adultes et la rapidité d'émergence ont été significativement affectées par la température. En effet, la longévité moyenne des adultes est de 18,22 jours à 15°C et chute à 4,58 jours à 35°C; la même tendance est observée pour le taux d'émergence qui passe de 87,56% à 15°C à 45,32% à 35°C. A hautes températures, *T. oleae* reste actif et peut être un bon candidat pour la lutte biologique contre les espèces de lépidoptères sous les climats chauds (températures proches de 30°C).

Mots clés: Elevage massif, *Ephestia kuehniella*, parasitoïdes d'œufs, stockage au froid, *Trichogramma oleae*

ملخص

الغربي، ناصر. 2014. تأثيرات مدة التخزين البارد ودرجة حرارة التربية على الخصائص البيولوجية للحشرة *Trichogramma oleae*. Tunisian Journal of Plant Protection 9: 143-153.

يعتبر طفيل البيض *Trichogramma oleae* مفيدا في برامج المكافحة البيولوجية لبعض الآفات الحشرية للزيتون. لذلك قمنا من خلال هذه الدراسة بتقييم تأثير سلسلة من فترات التخزين (من 1 الى 10 أسابيع) في درجة حرارية منخفضة (4 ± 1 °س مع رطوبة نسبية 75 ± 10 ٪ وظلام تام) وتأثير التربية في درجات حرارية مختلفة (من 15 الى 35 °س) على بعض الخصائص البيولوجية للسلالة التونسية للحشرة *T. oleae*. يبدو من خلال هذه الدراسة أن حوريات *T. oleae* قادرة على تحمل التخزين لمدة ثلاثة أسابيع كاملة دون ظهور أي تأثير سلبي على سرعة الانبثاق والخصوبة وطول مدة الحياة، وسرعة مشي البالغات)، بينما تأثرت كل هذه الخصائص البيولوجية بشكل ملحوظ بعد فترات تخزين أطول. وقد لوحظ أيضا أن نسبة التطفل لم تتأثر بصفة كبيرة عند الجيل الأول (F1) المتأتي من الحوريات المخزنة لمدة أقصاها ثلاثة أسابيع. كما أبرزت هذه الدراسة أن الترفيع في درجات حرارة التربية من 15 إلى 35 °س يؤدي إلى انخفاض هام في طول فترة التطور اليرقي والخصوبة ومدة تعمير الدبابير البالغة للحشرة *T. oleae* حيث تقلصت فترة التعمير من 18.22 يوم تحت 15 °س إلى 4.58 يوم تحت 35 °س. أما نسبة ظهور الحشرات الكاملة فكانت تقدر بـ 87.56 ٪ تحت 15 °س وانخفضت إلى 45.32 ٪ تحت 35 °س. مكن هذا البحث من استنتاج أن الطفيل *T. oleae* يبقى نشطا تحت درجات حرارة مرتفعة قريبة من 35 °س مما يجعله مرشحا جيدا للمكافحة قشريات الأجنحة تحت المناخات الدافئة (من 20 الى 30 °س).

كلمات مفتاحية: تخزين بارد، طفيل البيض، تربية مكثفة، *Ephestia kuehniella*، *Trichogramma oleae*

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