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Effect of cold storage on the performance of *Trichogramma bourarachae* (Pintureau and Babault) (Hymenoptera: Trichogrammatidae)

Khaled Abbes¹, Anis Zouba², Ahlem Harbi^{1,3} and Brahim Chermiti^{1*}

Abstract

In Tunisia, the use of the egg parasitoid trichogrammatid wasps to cope with several economic pests is gaining increasing attention. *Trichogramma bourarachae* (Pintureau & Babault) is among the *Trichogramma* wasps naturally present in Tunisia and regarded as a potential natural enemy to be used in biological control programs of many lepidopteran pests that cause economic losses. The propensity of *T. bourarachae* to cold storage with and without diapause induction was studied. The effect of this technique on its emergence rate and parasitic activity was investigated. The study showed that wasps of *T. bourarachae* treated at the induction temperature of 15 °C for 5 days can be stored at 4 ± 1 °C for more than 30 days without significant loss of their performances. By using this prestorage temperature, *T. bourarachae* could keep its emergence capacity (77.5%) up to 60 days of cold storage at 4 ± 1 °C. However, its parasitic activity decreased significantly (37.16%) after 45 days of cold storage. These results are promising for its mass production, transport, and release in the framework of national campaigns to control several agricultural pests of economic importance.

Keywords: Egg parasitoid, *Trichogramma bourarachae*, Cold storage, Parasitoid performance

Background

Trichogramma species (Hymenoptera: Trichogrammatidae) are among the most common natural enemies of several insect pests. They are used worldwide in biological control programs because of their ability to parasitize eggs of many economic lepidopterous pests of cultivated plants and their relatively easy mass rearing (Hassan, 1993). They have been used to treat millions of hectares of agricultural crops and forests against diverse insect pests (Li, 1994). In Tunisia, the use of trichogrammatid wasps is gaining increasing attention as they become officially used in the framework of national campaigns of integrated control of several economic

pests such as the carob moth *Ectomyelois ceratoniae* (Zeller), the pomegranate moth *Virachola livia* (Klug), and the invasive South American leaf miner *Tuta absoluta* (Meyrick). Among *Trichogramma* wasps naturally present in Tunisia, *Trichogramma bourarachae* (Pintureau & Babault) was reported in May 2002 in olive (*Olea europaea* L.) groves located in the region of Sfax (South East of Tunisia) (Herz et al., 2007). In 2010, it was also reported for the first time in the oases of the South Western Tunisia parasitizing eggs of *T. absoluta* and the carob moth *E. ceratoniae*. It is an abundant species in the Mediterranean area (Herz et al., 2007) and regarded as a potential natural enemy to be used in biological control programs of many lepidopterous economic crop pests.

Inundative releases of *Trichogramma* species as a biological control agents require their mass production

* Correspondence: chermiti54@yahoo.fr

¹Department of Plant Protection and Biological Sciences, High Agronomic Institute of Chott-Mariem, University of Sousse, Sousse, Tunisia
Full list of author information is available at the end of the article

(Tezze and Botto, 2004). Furthermore, release sites are often far from rearing facilities and these facilities are very scarce. Therefore, the development of adapted techniques of production, release, and storage is crucial to guarantee the flexibility, the profitability, and the efficiency of their mass rearing and to ensure their availability in sufficient numbers at the time of their release in the field (Leopold, 1998; Tezze and Botto, 2004; Ayvaz et al., 2008; and Lü et al., 2019). In particular, the cold storage technique which, in most cases, allows to delay wasps' eclosion without compromising their performance was studied for several *Trichogramma* species (Bradley et al., 2004; Ozder and Saglam, 2004; and Kumar et al., 2005) as it is important to investigate its effects on their biological performances and their propensity to cold storage (Tezze and Botto, 2004). Two main cold storage techniques have been used in mass rearing of *Trichogramma* spp., with and without diapause induction depending on the desired storage period and their sensitivity to diapause induction (Greenberg et al., 1996). Mass rearing of cold storage-adapted species can ensure high-quality parasitoids and contribute in reducing production and transport costs, while synchronizing releases of natural enemies with pest occurrence (Pitcher et al., 2002 and Colinet and Boivin, 2011).

In this context, the aim of this research was to study the ability of *T. bourarachae* to cold storage with and without diapause induction and to investigate the effects of this technique on its emergence rate and parasitic activity.

Materials and methods

Insect rearing

Adults of *T. bourarachae* used in this study were obtained from a laboratory colony, initially started with individuals collected in 2010 from parasitized eggs of *T. absoluta* infesting open filed tomato crops in the oases of Tozeur located in the South West of Tunisia. The parasitoid strain has been reared for many generations in the laboratory of entomology of the Technical Center of Dates (TCD) under controlled conditions at $25 \pm 1^\circ\text{C}$, $70 \pm 5\%$ RH and 16:8 (L:D) photoperiod on eggs of *Ephesia kuehniella* Zeller (Lepidoptera: Pyralidae) as a factitious host. *E. kuehniella* eggs were obtained from a laboratory rearing of the moth maintained on semolina under the same climatic conditions used for the wasp.

Effect of cold storage on *T. bourarachae*

Pieces of a white card (1×5 cm), containing one hundred fresh host eggs of *E. kuehniella* (< 1-day old), fixed with Arabic gum diluted to 30%, were placed separately in glass tubes (6 cm in length \times 1 cm in diameter) for 24 h with newly emerged *T. bourarachae* wasps (1-day old) under standard rearing conditions ($25 \pm 1^\circ\text{C}$ and 16:8 L:D

photoperiod). Each glass tube contained a droplet of honey solution to provide parasitoids with carbohydrates. After 24 h, adult parasitoids were removed from glass tubes. Carton cards containing the parasitized eggs were kept under standard rearing conditions until they reached the blackened stage (6th day). Three different cold storage procedures were tested: direct cold storage at $4 \pm 1^\circ\text{C}$ without diapause induction (prestorage), prestorage at 10°C for 5 days, followed by cold storage at $4 \pm 1^\circ\text{C}$ and prestorage at 15°C for 5 days, followed by cold storage at $4 \pm 1^\circ\text{C}$. The photoperiod used for the prestorage of parasitoids was L8:D16 because *Trichogramma* diapauses under short day length (Pizzol, 1978). Afterwards, the egg cards were stored for 0, 15, 30, 45, and 60 days at $4 \pm 1^\circ\text{C}$ in a refrigerator in a complete darkness. Similar temperatures (around 4°C) were used for the cold storage of other *Trichogramma* species (Ventura Garcia et al., 2002; Ma and Chen, 2006; and Ayvaz et al., 2008). Fifty egg cards were randomly assigned to each storage period for each cold storage procedure. At the end of each storage period, 10 randomly selected egg cards for each cold storage procedure were transferred into an incubator and maintained at standard rearing conditions ($25 \pm 1^\circ\text{C}$ and 16:8 L:D photoperiod) until the emergence of adult parasitoids.

The emergence rate was determined by dividing the number of parasitized eggs presenting emergence holes by the total number of parasitized eggs as a percentage for each tested cold storage procedure, for each storage period, and for each egg card. The impact of the cold storage procedures tested and the cold storage period on the performance of *T. bourarachae* females was studied as described by (Pizzol et al., 2010) with slight modifications. For each cold storage procedure and each cold storage period, 30 females (24-h old) were used to assess the parasitism and the mortality rates during the first 7 days after their emergence. Female parasitoids were individually isolated in glass tubes (6 cm in length, 1 cm in diameter) containing a carton card with 100 eggs of *E. kuehniella* and a droplet of honey solution as a food source. The egg cards were daily replaced by fresh ones for 7 days, and parasitized eggs were allowed to develop at 25°C and a 14:10 L:D photoperiod. The number of parasitized eggs and the number of dead females during the first 7 days after their emergence were determined. Furthermore, the reduction in parasitization of *T. bourarachae* on *E. kuehniella* eggs was estimated as a function of cold storage procedure and cold storage period (Siam et al., 2019).

Data analyses

Data sets were first tested for normality and homogeneity of variance using Kolmogorov Smirnov D test and Cochran's test, respectively. The effects of cold storage

procedure, of cold storage period, and potential interactions between these 2 factors on the emergence rate and parasitism rate of the wasp were estimated. For this, a factorial ANOVA was used. Subsequently, additional one-way ANOVA, followed by Duncan post hoc test for multiple comparisons inside the different cold storage procedure and cold storage period sub-datasets were carried out. All statistical analyses were performed using R project for statistical computing (R Core Team, 2019).

Results and discussion

The statistical analysis results are summarized in Table 1. The emergence rate of *T. bourarachae* adults varied significantly among cold storage procedures tested (significant cold storage procedure factor) and cold storage periods assessed (significant cold storage period factor). However, there was non-significant interaction between the cold storage procedure and cold storage period factors, i.e., cold storage procedure affects the emergence rate of the wasp the same way among cold storage periods. Concerning the parasitism rate ensured by *T. bourarachae*, it varied significantly among cold storage procedures tested (significant cold storage procedure factor) and cold storage periods assessed (significant cold storage period factor). Besides, there was a significant interaction between the cold storage procedure and cold storage period factors on the parasitism rate, i.e., cold storage procedure affects the parasitism rate of the wasp differently among cold storage periods.

The highest mean emergence rate (88.3%) was observed when *T. bourarachae* pupae were cold stored directly at $4 \pm 1^\circ\text{C}$. Lower mean emergence rate (87.0%) was observed when pupae were subjected to prestorage period at 15°C , while the lowest mean emergence rate (84.7%) was obtained when wasps were subjected to prestorage period at 10°C (Fig. 1). Concerning the effect of the storage period, overall, the emergences rate of *T. bourarachae* decreased when the storage period increased. Statistically similar emergence rates were observed after 0 and 15 days of conservation (93.8 and

92.6%) at $4 \pm 1^\circ\text{C}$ with, respectively. Lower emergence rates of 87.56, 82.36, and 77.00% were observed after 30, 45 and 60 days, respectively, of conservation at $4 \pm 1^\circ\text{C}$ (Fig. 1).

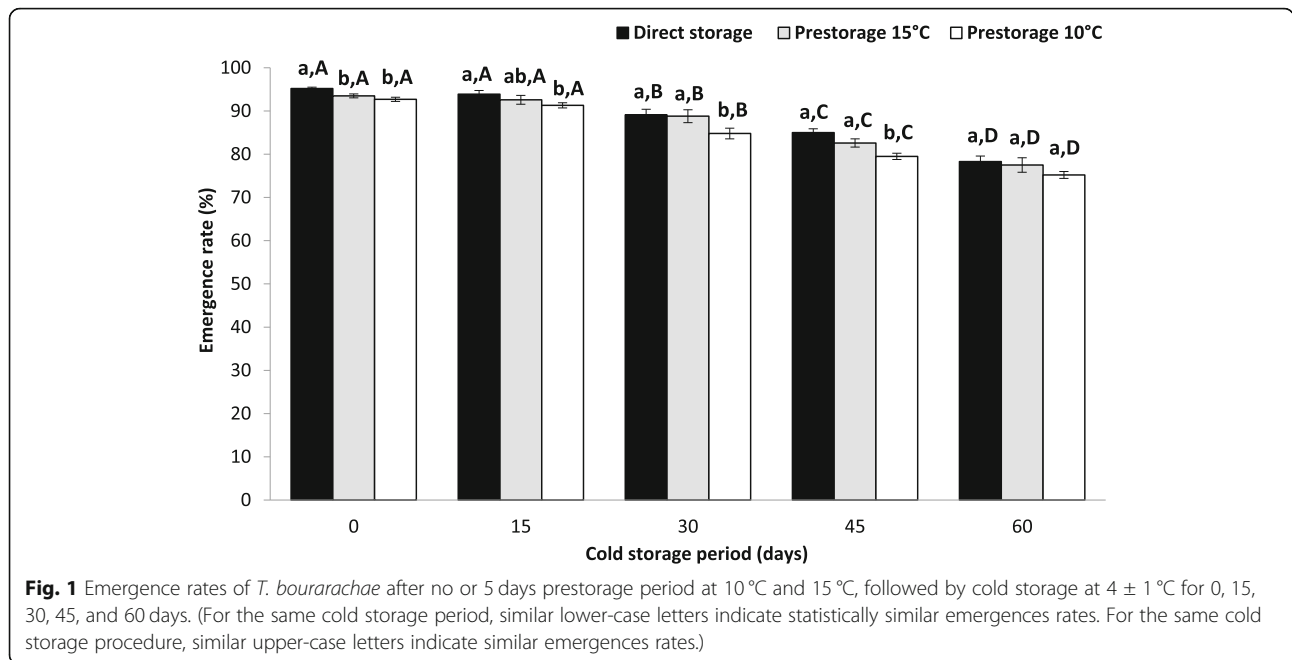
Parasitization of *T. bourarachae* on *E. kuehniella* eggs decreased with increasing of storage period (Fig. 2). The highest parasitization values were obtained by adults emerged from the pupae, which were not cold stored (0 days) and without statistical differences among all tested procedures with parasitism rates ranging from 56.86 ± 1.53 to 60.36 ± 1.41 eggs/female. The highest parasitization values after 15 days of cold storage were 44.26 ± 1.57 and 40.83 ± 1.82 eggs/female ensured by adult wasp emerged from pupae subjected to a prestorage period at 15°C and direct storage respectively without statistical differences. Whereas, significantly lower parasitization value of 31.86 ± 1.43 eggs/female was observed by wasps subjected to a prestorage period in 10°C . Statistically similar parasitization values were observed after 30 and 45 days of cold storage for wasps emerged from pupae subjected to prestorage period at 15°C . After 60 days of cold storage, parasitism activity was very low not exceeding 7 eggs/female in all evaluated storage procedures (Fig. 2).

Prolonged low temperature had a detrimental effect on the survival of *T. bourarachae* emerged from cold-stored pupae (Fig. 3). Mortality rates on the 7th day after adult's emergence increased substantially with increasing cold storage period, especially starting from day 15 using all storage procedures. Consequently, the estimated reduction in parasitization of *T. bourarachae* on *E. kuehniella* eggs varied as a function of cold storage procedure and cold storage period. The lowest parasitization reduction (39.64%) was observed at 0 days of direct storage, while the highest (95.37%) was noted after 60 days of storage, using the prestorage period of 15°C (Table 2). The highest mortality rate was registered after 60 days of cold storage in female wasp subjected to prestorage period at 10°C . Lower mortality rates of 56.67 and 50% were observed when

Table 1 Results of factorial ANOVA used to analyze the emergence rate and the parasitism rate of *T. bourarachae* on *E. kuehniella* eggs as function of cold storage procedure, cold storage period, and their interaction

Source of variation	Degrees of freedom	F	p value
Emergence rate			
Cold storage procedure	2	16.199	< 0.001***
Cold storage period	4	145.377	< 0.001***
Cold storage procedure × cold storage period	8	0.648	0.736
Parasitism rate			
Cold storage procedure	2	100.48	< 0.001***
Cold storage period	4	460.11	< 0.001***
Cold storage procedure × cold storage period	8	24.92	< 0.001***

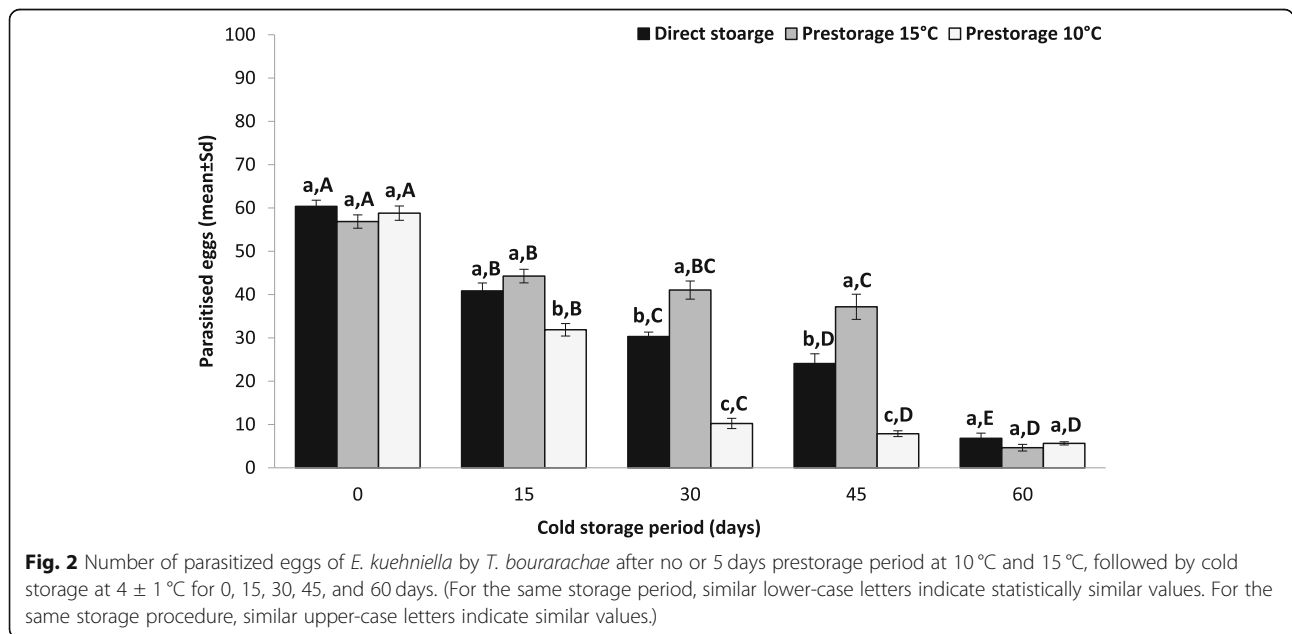
p values flagged with three stars (***) are highly significant at $\alpha = 0.05$

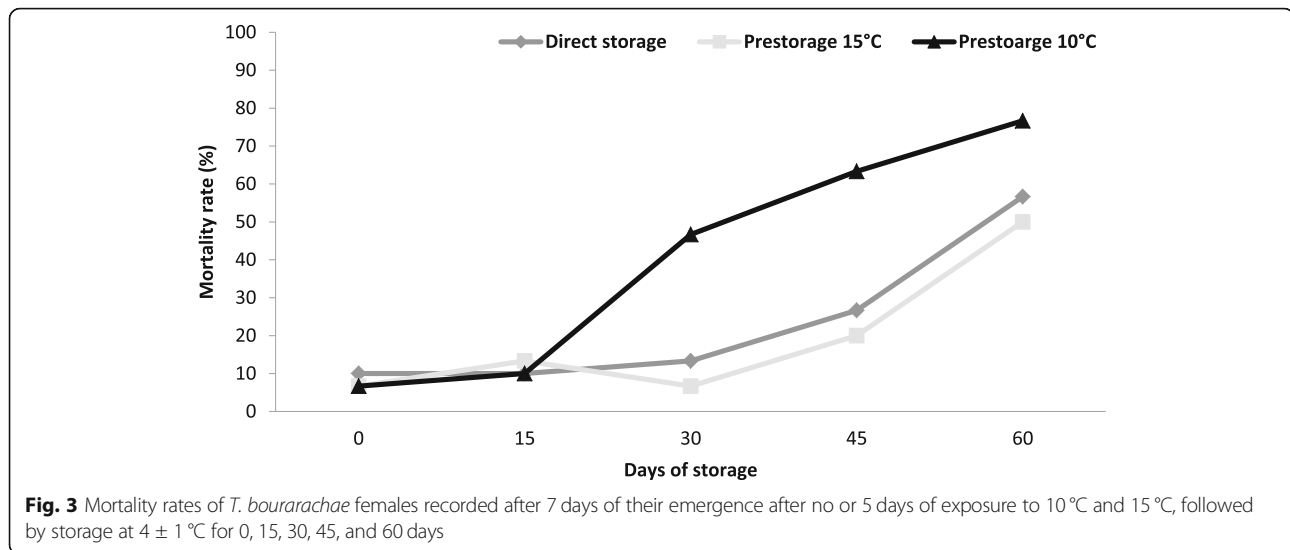


females were not subjected to prestorage period and prestored at 15 °C correspondingly (Fig. 3).

This study showed that *T. bourarachae* pupae are amenable to cold storage. However, prolonged cold storage periods can affect the rate of the emerged adults, as well as their parasitic ability. A reduction in the emergence rate due to the cold storage was also found with *T. nerudai* (Tezze and Botto, 2004) and *T. cordubensis* (Ventura Garcia et al., 2002). Similarly, a significant decline in parasitoid emergence rate was also reported by

Ozder and Saglam (2004) for *T. brassicae* and *T. evanescens* after storage at 4 °C for 3 weeks. The emergence rate of the Tunisian strain of *T. bourarachae* stored for 60 days at 4 °C without prestorage treatment (78.3%) was higher than that reported for *T. nerudai* and *T. cordubensis* when they stored at 3–4 °C for 50 and 60 days, respectively (Ventura Garcia et al., 2002; and Tezze and Botto, 2004). For almost all periods of cold storage, the emergence rates were similar for adults emerged from pupae treated at the induction temperatures 10 and





15 °C and for those stored directly at 4 ± 1 °C. This was noted in the case of study of Pizzol et al. (2010) who found that the induction temperature 15 °C was more favorable than 10 °C in terms of emergence rate of *T. cacoeciae* adults after prolonged periods of cold storage.

In the present study, stored parasitoids emerged 1 day later than in the control group and developmental time from pupa to adult emergence was 5 days for all the samples in the control group and 6 days for all the samples in each of the treatments. According to Tezze and Botto (2004), this difference could be due to the delay of normal metabolic activity after being cold stored for a prolonged period. Such a small difference of one more day in the developmental time is not very significant in mass rearing programs (Tezze and Botto, 2004).

Prolonged low temperature affected the longevity of *T. bourarachae* emerged from stored pupae. However, the percentage of survived parasitoid on the 7th day after adults' emergence remained high, when pupae were subjected to the prestorage of 15 °C and stored for 45 days at 4 ± 1 °C. This finding corroborates with those of Jalali and Singh (1992) who reported that longevity of *T. achaea* Nagaraja and Nagarakati, *T. chilonis* Ishii, and *T. japonicum* Ashmead declined after cold storage for 2 weeks at 2–5 °C.

In the present study, the parasitic ability of *T. bourarachae* females stored directly at 4 ± 1 °C, without prestorage treatment, decreased with increasing the storage period beyond 30 days. A reduction in the parasitic ability due to the direct cold storage was also reported by Ayvaz et al. (2008) for *T. evanescens*. However, Piao et al. (1992) found no difference in post-storage parasitism rate for *T. dendrolimi* Matsumura females stored for up to 30 days at 3–5 °C.

Obtained data showed that *T. bourarachae* pupae pre-stored at the induction temperature of 15 °C can be cold stored at 4 ± 1 °C up to 30 days without much loss of performance. Despite some decrease in the parasitism rate of the adults emerged from pupae treated at 15 °C and stored for 45 days at 4 ± 1 °C, the mean number of parasitized eggs was high (37 parasitized eggs/female). Similar results were reported for *T. cacoeciae* by Pizzol et al. (2010) with the difference that the induction temperature of 10 °C was better than 15 °C in preserving the parasitic ability of the wasp after prolonged periods of cold storage.

Conclusion

The best procedure for *T. bourarachae* strain to be cold stored was by diapause induction at 15 °C, followed by a

Table 2 Estimated reduction in parasitization (%) of *T. bourarachae* on *E. kuehniella* eggs as function of cold storage procedure and cold storage period

Cold storage periods (day)	Direct storage	Prestorage 10 °C	Prestorage 15 °C
0	39.64	41.2	43.14
15	59.17	68.14	55.74
30	69.7	89.77	58.77
45	75.94	92.14	62.84
60	93.2	94.37	95.37

cold storage at $4 \pm 1^\circ\text{C}$ up to 30 days. This cold storage procedure allowed high emergence rates of the wasp and satisfactory parasitism rates. Mass rearing and releases of the wasp can greatly contribute to controlling several economic agricultural pests in Tunisia.

Abbreviation

TCD: Technical Center of Dates

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Authors' contributions

BC designed the experiment, AZ conducted the essay, and KA and AH analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

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Competing interests

The authors declare that they have no competing interests.

Author details

¹Department of Plant Protection and Biological Sciences, High Agronomic Institute of Chott-Mariem, University of Sousse, Sousse, Tunisia. ²Centre Technique des Dattes, Tozeur, Tunisia. ³Unidad Asociada de Entomología IVA-CIB CSIC, Centro de Protección Vegetal y Biotecnología, Instituto Valenciano de Investigaciones Agrarias (IVA), Ctra. Moncada a Naquera km 4.5, 46113 Moncada, Spain.

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References

- Ayvaz A, Karasu E, Karasu E, Karaborklu S, Tunçbilek AS (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
- Bradley JR, Thomson LJ, Hoffmann AA (2004) Effects of cold storage on field and laboratory performance of *Trichogramma caveriae* (Hymenoptera: Trichogrammatidae) and the response of three *Trichogramma* spp. (*T. caveriae*, *T. nr. brassicae*, and *T. funiculatum*) to cold. *J Econ Entomol* 97:213–221
- Colinet H, Boivin G (2011) Insect parasitoids cold storage: a comprehensive review of factors of variability and consequences. *Biol Control* 58:83–95
- Core Team R (2019) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria <https://www.R-project.org/>
- Greenberg SM, Nordund DA, King EG (1996) Mass production of *Trichogramma* spp.: experience in the former Soviet Union, China, United States and Western Europe. *Biocontrol News Inform* 17(3):51–60
- Hassan SA (1993) The mass rearing and utilization of *Trichogramma* to control lepidopterous pests: achievements and outlook. *Pestic Sci* 37(4):387–391
- Herz A, Hassan SA, Hegazi E, Khafagi WE, Nasr FN, Youssef AI, Agamy E, Blibech I, Ksentini I, Ksantini M, Jardak T, Bento A, Pereira JA, Torres L, Souliotis C, Moschos T, Panos M (2007) Egg parasitoids of the genus *Trichogramma* (Hymenoptera, Trichogrammatidae) in olive groves of the Mediterranean region. *Biol Control* 40:48–56
- Jalali SK, Singh SP (1992) Differential response of four *Trichogramma* species to low temperatures for short term storage. *Entomophaga* 37:159–165

- Kumar P, Shenmar M, Brar KS (2005) Effect of low temperature storage on the efficiency of three species of trichogrammatids. *J Biol Cont* 19:17–21
- Leopold RA (1998) Cold storage of insects for integrated pest management. In: Hallman GJ, Denlinger DL (eds) *Temperature Sensitivity in Insects and Application in Integrated Pest Management*. Westview Press, Boulder, CO, pp 235–267
- Li LY (1994) Worldwide use of *Trichogramma* for biological control on different crops: a survey. In: Wajnberg E, Hassan SA (eds) *Biological Control with Egg Parasitoids*. CAB International, Wallingford, UK, pp 37–53
- Lü X, Han S, Li J, Liu J, Li Z (2019) Effects of cold storage on the quality of *Trichogramma dendrolimi* Matsumura (Hymenoptera: Trichogrammatidae) reared on artificial medium. *Pest Manag Sci* 75:1328–1338
- Ma CS, Chen YW (2006) Effects of constant temperature, exposure period, and age on diapause induction in *Trichogramma dendrolimi*. *Bio Control* 36:267–273
- Ozder N, Saglam O (2004) Effect of short-term cold storage on the quality of *Trichogramma brassicae*, *T. cacoeciae*, and *T. evanescens* (Hymenoptera: Trichogrammatidae). *Great Lakes Entomol* 37:183–187
- Piao YF, Lin H, Shi GR (1992) Quality control of the physique of mass-reared *Trichogramma*. *Plant Prot* 18:28–29
- Pitcher SA, Hoffmann MP, Gardner J, Wright MG, Kuhar TP (2002) Cold storage of *Trichogramma ostrinia* reared on *Sitotroga cerealella* eggs. *Bio Control* 47: 525–535
- Pizzol J (1978) La diapause chez *Trichogramma evanescens* Westwood (Hym., Trichogrammatidae), écotype moldave parasite oophage de la Pyrale du maïs. Diplôme, de l'Ecole Pratique des Hautes Etudes. Institut de Montpellier, 78 pp.
- Pizzol J, Pintureau B, Khoualdia O, Desneux N (2010) Temperature dependent differences in biological traits between two strains of *Trichogramma cacoeciae* (Hym. Trichogrammatidae). *J Pest Sci* 83:447–452
- Siam A, Zohdy NZM, ELHafez AMA, Moursy LE, Sherif HAEL (2019) Effect of different cold storage periods of rearing host eggs on the performance of the parasitoid *Trichogramma evanescens* (Westwood) (Hymenoptera: Trichogrammatidae). *Egypt J Biol Pest Control* 29(34):1–4
- Tezze AA, Botto EN (2004) Effect of cold storage on the quality of *Trichogramma nerudai* (Hymenoptera: Trichogrammatidae). *Biol Control* 30:11–16
- Ventura Garcia P, Wajnberg E, Pizzol J, Oliveira MLM (2002) Diapause in the egg parasitoid *Trichogramma cordubensis*: role of temperature. *J. Insect Physiol* 48:349–355

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