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RESEARCH

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Reproductive performance and functional response of *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae) obtained from cold-stored red-eyed pupae

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Abstract

Background: Cold storage of reared natural enemies is important in terms of planning the release time and quantity, eliminating unpredicted demand increases, and reducing production costs. However, the tolerance of reared natural enemies at low temperatures varies depending on the species and needs to be determined. *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae) is one of the most important natural enemies used in biological control of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) in greenhouses.

Results: In a laboratory study, longevity, parasitism capacity and functional response of *E. mundus* adults obtained from 8-days cold-stored red-eyed *E. mundus* pupae at 10 °C with 45 ± 5% RH conditions were determined. Mean longevity obtained from stored *E. mundus* pupae of female and male were 23.6 and 16.2 days, respectively. However, parasitism capacity was negatively affected so that the total mean number of immature *B. tabaci* parasitized by an *E. mundus* female obtained from cold-stored pupae (13.6) was statistically lower than that obtained from the colony (26.8) reared at room temperature. Adults obtained from both non-stored and stored *E. mundus* pupae exhibited a type II functional response to increasing host density. Although cold storage did not alter the type of functional response, it negatively affected the maximum attack rate (*a*) and handling time (*Th*) of the parasitoid. The lowest maximum attack rate (1.56) and highest handling time (0.059) were obtained for adults of cold-stored *E. mundus* pupae.

Conclusions: The results obtained may contribute to the augmentative biological control of *B. tabaci* in greenhouses.

Keywords: *Eretmocerus mundus*, *Bemisia tabaci*, Cold storage, Longevity, Parasitism capacity, Functional response

Background

The sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is one of the most prominent pests in agricultural production systems including greenhouses (Oliveira et al. 2001). Chemical control is the primary method preferred for the management of the pest. Insecticide applications that are not done properly can cause serious human and environmental health problems. On the other hand, successful biological control treatments could avoid the negative effects such as residue and resistance caused by chemicals (Kazak et al. 2020).

Many natural enemies are mass-reared and marketed by various companies to be used in the biological control of *B. tabaci*, especially in greenhouses (Gerling et al. 2001). *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae) is one of the most important parasitoids used in the biological control of *B. tabaci.* It is a parasitoid native to the Mediterranean region and has a significant commercial value in the augmentative biological

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control programs of *B. tabaci* (van Lenteren 2012). Its commercial use began in Europe in 2001 and it is ranked 23rd among the most used natural enemies in the world (van Lenteren 2012). Parasitism rates by this parasitoid reached up to 90% (in greenhouse), and it mostly prefers the 1st and 2nd instars of *B. tabaci* for parasitism (Stansly et al. 2004; Gerling and Foltyn 2009).

The increase in integrated pest management applications and awareness among producers has also increased the demand for natural enemies. Accordingly, the importance of mass production of natural enemies has also increased. However, a short life span and difficulty in transportation due to temperature and humidity demands are reported to be the most notable challenges encountered in mass production of natural enemies (Venkatesan et al. 2000). Cold-storage of augmentative natural enemies is important in terms of planning the release time and quantity, eliminating unpredicted demand increases, and reducing production costs. Therefore, many studies have been carried out on the storage technique of biological control agents have shown that short-term cold storage has different effects on predator/parasitoid biology and reproduction (van Lenteren and Woets 1988; Tauber et al. 1993; van Lenteren and Tommasini 2002; Nadeem et al. 2014).

Since insects are poikilothermic, they can already survive at low temperatures where climate are not optimal. Through manipulation of this feature of insects, mass-produced natural enemies can be stored for use when needed. However, the tolerance of natural enemies used in biological control to low temperatures varies depending on the species and needs to be determined accordingly. Therefore, this study was conducted to evaluate the performance of adults obtained from cold-stored *E. mundus* pupae under laboratory conditions.

Methods

Host plant, *Bemisia tabaci* and *Eretmocerus mundus* rearing

Cotton (*Gossypium hirsutum* L.) plant was used for mass rearing of *B. tabaci* MEAM1 (Middle East–Asia Minor 1) and *E. mundus*, and for setting up the experiments. The host plant rearing was carried out in a controlled room at 25 ± 2 °C with 70 ± 5% RH conditions in pots filled with fertile soil. For the *E. mundus* colony, the parasitoid pupae were obtained from cotton fields of the Çukurova University, Faculty of Agriculture, Research and Implementation Sites, Adana, Turkey. Dark parasitoid subtraction boxes containing glass tubes were used for obtaining adult parasitoids (Kazak et al. 2020). For the requested parasitoid population level, one hundred recently hatching adult parasitoids were afterward released into cages (50 × 50x70 cm), which had 8 whitefly-infested cotton plants. The parasitoid colony was repeatedly provided with *B. tabaci* cotton plants acquired from the *B. tabaci* rearing cages, during the experiment (10 generations). For this aim, separate whitefly rearing cages (50 × 50x70 cm) were prepared with cotton plants in insect rearing rooms (25 ± 2 °C, 70 ± 5% RH, 16L: 8D). The parasitoid, *E. mundus* was confirmed by using morphological characters such as: antenna, colorization of the first 2 abdominal segments and ovipositor structure (Sharaf 1982; Kim and Heraty 2012).

Longevity of *Eretmocerus mundus* adults obtained from cold-stored pupae

Red-eyed *E. mundus* pupae were used in the experiment. For obtaining red-eyed pupae from the parasitoid colony, cotton leaves with parasitized *B. tabaci* were examined under a stereomicroscope. Then, selected 5 red-eyed *E. mundus* pupae, with mouthparts in the up situation, were stuck on one-sided sticky paper strips (1 × 5 cm), using an insect pin and a fine brush (Kazak et al. 2020). The paper strips were then settled in Eppendorf tubes and located in a controlled environmental test cabinet (NUVE®TK252) at 10 °C with 45 ± 5% RH, 16L:8D conditions for 8-day storage periods (Kazak et al. 2020). Totally, 100 pupae were stored. The storage conditions used in this study were selected according to the results of a previously published study by Kazak et al. (2020). After 8 days, 50 stored *E. mundus* pupae, 5 in one Eppendorf tube (1.5 ml), were transferred to a controlled cabinet at 25 °C with 75 ± 5% RH, 16L: 8D for determining adult longevity. A similar number of *E. mundus* pupae (50) obtained from the culture were also transferred to the chamber and evaluated as a control. Before adult emergence, the pupae transferred to new Eppendorf tube individually and a small drop of honey was smeared at the cap of the tube for feeding the emerged parasitoid adults. The emerged parasitoids were observed daily until the last adult was dead. Not all the stored pupae succeeded to the adult stage. Therefore, the mean longevity was calculated according to data obtained from at least 19 adults for each stored and control group for females and males separately. Males and females were separated according to antennal clava. Females have antennal clava, which are 5 times as long as wide, while those of males are 6 times as long as wide (Kim and Heraty 2012).

Parasitism performance of *Eretmocerus mundus* adults obtained from cold-stored pupae

The 2nd instar *B. tabaci* nymphs was used in the study. To obtain the desired nymph instar, clean cotton plants were kept in the whitefly rearing cages, and the females were allowed to lay eggs. After 24 h, the whiteflies were removed and the plants were transferred to a climate room with a temperature of 25 ± 1 °C and 70 ± 5% RH, 16L: 8D photoperiod. The nymphs were monitored until the 2nd instar (13–14 days). The cotton leaves infested with 2nd instar nymphs were cut at (3 cm in diameter) and placed in a 5-cm-diameter Petri dish containing water agar with the upper surface facing downwards. The leaf discs were checked under a stereomicroscope, and each disc was arranged to have 20 2nd instar *B. tabaci* nymphs. The Petri dishes were then covered with a lid that allows ventilation through a fine mesh net. Subsequently, a pair of newly emerged *E. mundus* adults obtained from cold-stored and un-stored red-eyed pupae as described above was transferred to the prepared Petri dishes with the aid of a sucking tube. The pairs were transferred to a new Petri dish at 2-day intervals for 8 days. Totally 80 2nd instar *B. tabaci* nymphs were exposed to the female during this period. The test units were kept at a controlled environmental chamber until the first sign of parasitism, displacement of host mycetomes, 7–9 days after oviposition (Mohammed and Karut 2021). The parasitized and un-parasitized nymphs were then separated with the aid of a stereomicroscope to determine the parasitoid performance. Fifteen pairs, all obtained from the same rearing generation were used for each treatment. Feeding behavior of adult *E. mundus* on immature stages of *B. tabaci* was not evaluated in this study.

Determining functional response parameters of *Eretmocerus mundus* adults obtained from cold-stored pupae

In the functional response study, cotton leaf discs with 5, 10, 20, 40 and 80 of 2nd instar *B. tabaci* nymphs prepared as described above were used. A pair of newly emerged adult parasitoids that emerged from cold-stored and un-stored red-eyed *E. mundus* pupae were released into the prepared Petri dishes. After 24 h, adults were removed, and the Petri dishes were kept in the controlled cabinet (with a temperature of 25 ± 1 °C, 70 ± 5% RH and 16L: 8D photoperiod) until the first sign of parasitism. The experiments were repeated 15 times for each host density separately for adults obtained from cold-stored and un-stored red-eyed *E. mundus* pupae.

Data analysis

Effect of cold storage on longevity and the parasitism capacity of *E. mundus* were compared by using t test. Before analysis, the data were first subjected to logarithmic and angular transformation for normalization.

The type of functional response of parasitoid was determined in two-step data analysis (Juliano 2001). In the first step, the type was determined by a logistic regression of the proportion of the host parasitized (N_a/N_0) in relation to the initial host density (N) (Eq. 1). The maximum likelihood test was used to estimate the parameters P_0, P_1, P_2 and P_3. In all cases, since ${N_o}^3$ were not significant, cubic confidents were removed from the formula, and the data were re-subjected to the logistic regression. If linear coefficient P_1 is significantly negative, a type II functional response is evident, whereas a positive linear parameter indicates a type III functional response (Juliano 2001).

In the second step, the handling time (Th) and the attack rate (α) coefficients of a type II response were estimated using the Rogers (1972) random parasitoid equation (Eq. 2), since the initial host densities were provided without replacement. Nonlinear least square regression was used to estimate the parameters of the Rogers (1972) random parasitoid Eq. (2). All analyses were performed using Microsoft Excel and SPSS 23.0 (SPSS, Chicago IL, USA).

$$\frac{N_a}{N_0} : \frac{\exp(P_0 + P_1N_0 + P_2{N_0}^2 + P_3{N_0}^3)}{1 + \exp(P_0 + P_1N_0 + P_2{N_0}^2 + P_3{N_0}^3)} \tag{1}$$

N_a = number of host parasitized; N_0 = host density and P_0, P_1, P_2 and P_3 are the intercept, the linear, the quadratic and the cubic factor,

$$\text{Npar} : N\left[1 - \exp\left(-\frac{\alpha T}{1 + \alpha \text{Th} N}\right)\right] \tag{2}$$

where Npar is the number of parasitized host, N is initial host density, α is attack rate, Th is handling time (h) and T is total time available for searching during the experiment (h).

Results

The mean longevity of females and males obtained from stored red-eyed *E. mundus* pupae was shorter but non-statistically different from adults obtained from un-stored *E. mundus* pupae (Female: *df*: 22, *t*: 1.92, *P*: 0.06; male: *df*: 18, *t*: 1.26, *P*: 0.22). While the mean longevity periods of unmated females and males obtained from un-stored pupae were 25.7 ± 0.80 and 17.6 ± 0.70 days, respectively, these values were 23.6 ± 0.69 and 16.2 ± 0.67 days for females and males obtained from stored *E. mundus* pupae, respectively (Fig. 1). The total mean number of immature *B. tabaci* parasitized by *E. mundus* females obtained from stored pupae (13.6 ± 2.54) during 8 days was statistically lower than the ones parasitized by the females obtained from the unstored colony (26.8 ± 2.79) (*df*: 41, *t*: 3.42, *P*: 0.001) (Fig. 2).

The mean parasitism rates of the females obtained from un-stored and stored *E. mundus* pupae varied according to host density, and parasitism rates declined when host densities were increased. The rates ranged from 18.01 to 68.6 and 16.6–55.7% for the females obtained from un-stored and stored pupae, respectively (Fig. 3). In low host

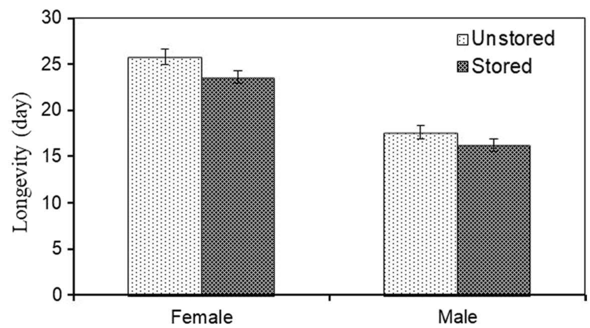

Fig. 1 Mean (±SE) longevity of *Eretmocerus mundus* adults obtained from unstored and eight-day-stored pupae. Means are not significantly different based on *t* test (> *P*: 0.05)

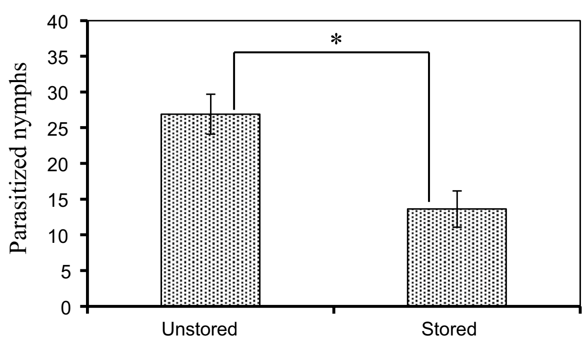

Fig. 2 The total mean (±SE) number of immature *Bemisia tabaci* parasitized by *Eretmocerus mundus* females obtained from unstored and eight-day-stored *E. mundus* pupae. The asterisk indicates significant difference based on *t* test ($P<0.05$)

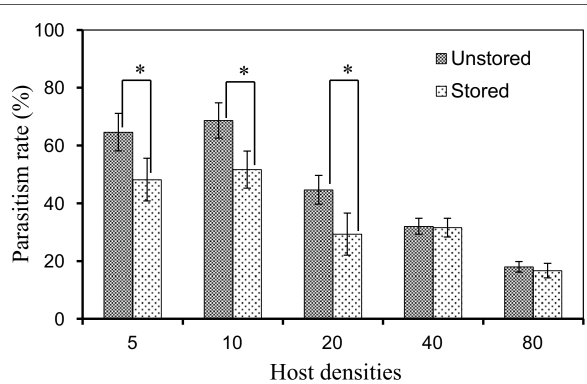

Fig. 3 Mean (±SE) parasitism rates of *Eretmocerus mundus* females obtained from unstored and eight-day-stored pupae to the different 2nd instar nymph densities of *Bemisia tabaci*. The asterisk indicates significant difference based on *t* test ($P<0.05$)

densities, the rates were found to be higher than in high host densities, and the differences among treatments were statistically significant when 5, 10 and 20 host

Table 1 Estimated coefficients by logistic regression of proportion of hosts parasitized by adult *Eretmocerus mundus* obtained from unstored and eight-day-stored pupae in relation to initial *Bemisia tabaci* nymph densities

	Parameter	Estimates (±SE)	χ^2	*P*
Unstored	P_0*	1.0135 (±0.1903)	28.34	<0.0001
	P_1	− 0.0553 (±0.0096)	32.59	<0.0001
	P_2	0.0002 (±0.00009)	9.408	0.002
Stored	P_0	0.6092 (±0.1744)	12.20	<0.0001
	P_1	− 0.0472 (±0.009)	26.62	<0.0001
	P_2	0.0002 (±0.00009)	6.969	0.008

[*] P_0, P_1, and P_2 are the intercept, the linear, and the quadratic factors, respectively

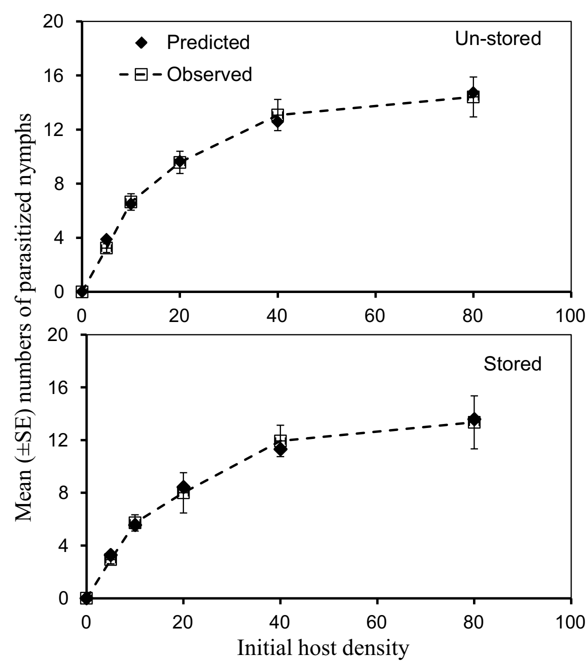

Fig. 4 Functional response curves of *Eretmocerus mundus* adults obtained from unstored and eight-day-stored pupae to the different 2nd instar nymph densities of *Bemisia tabaci*

densities were provided (five: *df*:33, *t*: 1.95, *P*: 0.05; ten: *df*: 30, *t*:1.69, *P*: 0.02; twenty: *df*: 24, *t*: 1.71, *P*: 0.03) (Fig. 3).

Linear coefficients and P_1 values were found to be significantly different at the 2 treatments (Table 1). The proportion of parasitism increased with increasing host density for parasitoids obtained from both un-stored and stored pupae (Fig. 4). The logistic regression analysis showed that parasitoids obtained from both un-stored and stored pupae showed a type II functional response by a negative P_1 parameter (Fig. 4 and Table 1). The functional response of adult female parasitoid to whitefly immature at 2 different treatments well fitted to the random parasitoid equation (Rogers 1972). The higher attack rate and the shorter handling time were found to be 2.569 and 0.057 for adults from un-stored pupae treatment (Table 2).

Discussion

Storage at 10 °C had a non-negative effect on the longevity of adult parasitoids that emerged from cold-stored pupae. The mean longevity of *E. mundus* females and males obtained from un-stored and stored pupae in this study was higher than the results published earlier (Qiu et al. 2004; Lopez and Botto 2005; Urbaneja et al. 2006; Zandi-Sohani et al. 2009). The values of this parameter reportedly ranged between 3.9 and 10.5 days depending on the temperature and nutritional quality of the food which parasitoids were fed (Zandi-Sohani et al. 2009). The decline in the longevity of adults from stored treatments at low temperature was also reported for a variety of hymenopterous parasitoids (Kidane et al. 2015; Anwar et al. 2016; Yan et al. 2017). Ismail et al. (2012) attributed this to the amount of energy reserves remaining inside the body after storage in both sexes. They speculated that there was a positive relationship between survival and the amount of lipid reserves accumulated from the host during larval development.

Cold storage had negatively affected the parasitism capacity of *E. mundus* by causing almost a 50% reduction in the number of parasitized whitefly nymphs. Similar to obtained results, Luczynski et al. (2007) reported that storage at 7 and 12 °C had a negative effect on the parasitism capacity of *Eretmocerus eremicus* Rose & Zolnerowich and *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae), while Lopez and Botto (2005) found a reduction on 1st day parasitism of adult *En. formosa* after > 14 days of storage and reported that females stored at 4.5 °C did not lay eggs after a 28-day storage period. In addition to the above-mentioned studies, it was reported that the parasitism capacity of different species of parasitoids decreases depending on the temperature and storage time (Al-Tememi and Ashfaq 2005). On the contrary, it was reported that storing of the egg-parasitoid, *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae) at 4 and 6 °C for 3 weeks did not negatively affect its parasitism ability (El-Gawad et al. 2010). Similarly, Lopez and Botto (2005) also observed non-statistical difference among the numbers of hosts parasitized by *Eretmocerus corni* Haldeman individuals stored for 7, 14, 21, and 28 days at temperatures between 4.5 and 11.5 °C.

In the functional response study, both *E. mundus* adults obtained from un-stored and 8-day-stored pupae exhibited a type II response to increasing host density. This is in agreement with the findings of previous studies conducted with *E. mundus* (Awadalla et al. 2014; Najem and Al-Rubeae 2015; Xu et al. 2016).

Although cold storage did not change the model of functional response of the parasitoid in the present study, it did negatively affect the maximum attack rate and handling time of *E. mundus*. The lower maximum attack rate of 1.56 was observed for *E. mundus* adults obtained from cold-stored pupae. This may be attributed to the increase in energy necessity and gradual weakness of the individual during metamorphosis in the storage period. Therefore, accumulation of toxic metabolites, oxidative stress caused by a buildup of reactive oxygen, and extraction of energy reserves have been suggested as probable causes for decreasing suitability of individuals after extended exposures to cold temperatures (Colinet and Boivin 2011).

The results of this study showed that parasitism capacity, and functional response parameters of adults obtained from 8-day-stored *E. mundus* pupae were negatively affected by the storage. However, Kazak et al. (2020) reported that cold storage did not negatively affect the parasitism ability of a native strain (Adana, Turkey) of *E. mundus*, suggesting that the parasitoid could be used successfully in the biological control of *B. tabaci* in greenhouse-grown tomato plants. This may be due to the fact that F_1 individuals obtained from parents were not affected by cold storage, although it was not tested in this study.

Table 2 Attack rate (*a*), handling time (Th), and standard error values of the random parasitoid equation for adult *Eretmocerus mundus* obtained from unstored and eight-day-stored pupae at 10 °C degrees

	$a \pm$ SE (95% CL)*	Th ± SE (95% CL)	R^2
Unstored	2.569 ± 1.377 (0.705–4.433)	0.057 ± 0.006 (0.045–0.068)	0.527
Stored	1.560 ± 0.854 (0.568–2.553)	0.059 ± 0.009 (0.042–0.076)	0.357

* CL: Confidence limits were obtained based on bootstrap method with 10,000 iterations

Conclusions

It was concluded that although a decrease in the parasitism ability of adults obtained from cold-stored pupae was detected, this can be tolerated in subsequent generations. Therefore, to increase the success of augmentative biological control of *B. tabaci*, additional storage studies of *E. mundus* should be conducted at different temperatures and humidity levels until optimization of appropriate conditions is attained.

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Author contributions
BK: Investigation. MMK: Investigation. KK: Methodology, Supervision, Writing—Original draft preparation. 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.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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