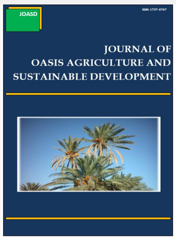

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Suitability of *Ectomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae) as host for two local *Trichogramma* species (Hymenoptera: Trichogrammatidae) and possible implications on biological control programs

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Abstract

In this study, laboratory tests were conducted to evaluate the suitability of *Ectomyelois ceratoniae* (Zeller) eggs as host for the development of two *Trichogramma* species: *Trichogramma cacoeciae* (Marchal) and *Trichogramma bourarachae* (Pintureau and Babault) (Hymenoptera: Trichogrammatidae) collected in Tunisia and reared on *Ephestia kuehniella* Zeller eggs. We also studied effects of five different temperature regimes (15, 20, 25, 30 and 35°C) on the biological traits of these two parasitoids. Results showed that eggs of *E. ceratoniae* were more suitable for the development of *T. bourarachae*. The number of parasitized eggs (15.46±2.04) as well as the emergence rate (95.78±5.10) was significantly higher compared to *T. cacoeciae*. For both *Trichogramma* species, the highest parasitism rate was observed at 30°C. At 35°C, the mean number of parasitized eggs (32.06±13.32) and the longevity (4.03±0.56) of *T. bourarachae* females were significantly higher compared to *T. cacoeciae*. The developmental time of both *Trichogramma* species was shorter at higher temperatures. The emergence rates were similar and relatively high in all tested temperature ranging from 80.48% to 97.92% for *T. bourarachae* and from 92.03% to 99.33% for *T. cacoeciae*.

1. INTRODUCTION

Date palm is one of the most economically important fruit trees grown in southern Tunisia representing 18% of the national tree production (OTTD/GTZ, 2010). It generates income for local population and contributes to the economical balance of the country as fruits and transformed products are exported. Nevertheless, in Tunisia, date palm cultivations are harshened by drought, salinization, genetic erosion as well as the spread of disease and pests.

In particular, Ectomyelois ceratoniae Zeller (Lepidoptera: Pyralidae), commonly known as the carob moth, causes significant damage on many fruit species in different regions around the world (Botha et al., 2004) under field and storage conditions (Mehrnejad, 1995). In Tunisia, E. ceratoniae is regarded as the most important phytosanitary problem of date production (Dhouibi, 1989). Date infestation in the field or storage facilities depreciates significantly fruit quality and compromises exports particularly in the case of Deglet Noor variety (Khoualdia, 2006). Furthermore, few insecticides are available for efficient control of this pest (Vetter et al. 1997). Currently, nonchemical methods are used and recommended to control this pest (Sarjami et al., 2009). Among natural enemies of E. ceratoniae, egg parasitoids of the genus Trichogramma (Hymenoptera: Trichogrammatidae) are widely used in the biological control strategies of Lepidoptera crop pests (Li, 1994), due to their economically reasonable mass rearing procedure and their potential to parasitize many important agricultural pests before even hatching and causing feeding damage (Hassan, 1998; Ulrichs & Mewis, 2004). Currently about twelve Trichogramma species are used in biological control programs against crop pests, particularly against Lepidoptera (Kumar et al., 2009) with 32 million hectares being treated worldwide (Li, 1994). Different native species of Trichogramma have been evaluated for controlling the carob moth (Moezipour, 2006). In particular, Trichogramma cacoeciae (Marchal) is a thelytokous parasitoid (males come from fertilized eggs and females come from unfertilized eggs) which is widely used as biological agent against various pest species (Pinturea, 1997). It was reported to be omnipresent in many countries in Europe (Volkoff & Daumal, 1994 ; Pintureau et al. 1999 ; Vavre et al., 2004). In Tunisia, T. cacoeciae was reported in olive (Olea europaea L.) groves in Sfax (South East of Tunisia) (Herz et al., 2007), in pomegranate (Punica granatum L.) orchards in Gabes (South East of Tunisia) (Ksentini et al., 2010) and it is widely present in the oases of the South West of Tunisia where extreme weather conditions are prevalent (Zouba et al., 2013). Trichogramma bourarachae (Pintureau and Babault) is an arrhenotokous parasitoid known by its resistance to high temperatures. Herz et al. (2007) reported that this Trichogramma species is generally abundant and typical for the Mediterranean area. According to the same author, T. bourarachae was obtained from the eggs of many Lepidoptera pests in this area. One of the corner stones of a biological control programs using parasitoids is the selection of species and strains that are able to control the pest by reducing damage below the economic threshold (Samara et al. 2008). Therefore, laboratory studies are essential to assess the biological characteristics of the parasitoid and its interaction with the targeted host pest species (Bourchier & Smith, 1996) prior its release in targeted crops. In case of Trichogramma species, their potential in controlling insect pests is usually assessed based on fecundity, emergence rate from host eggs, longevity and host suitability which is one of the factors that affect the development of the parasitoid within the host (Roriz et al., 2006; Samara et al., 2011). Johannes et al. (2001) defined the host suitability by the ability of parasitoid species to accept and to develop in the respective host.

Among abiotic factors that could greatly influences parasitism in Trichogramma species, temperature is an important parameter that can affect biological characteristics namely developmental rate, fecundity and longevity (Gunie & Lauge, 1997; Prasad et al., 1999) and consequently, the distribution and abundance of the parasitoid in the field (Maceda et al., 2003). Subsequently the effect of temperature on the performance of parasitoids should be addressed before their use in biological control strategies.

The aim of this work is to evaluate the suitability of E. ceratoniae eggs as host for the local strains of T. cacoeciae and T. bourarachae and to assess the effect of five different temperature regimes (15, 20, 25, 30 and 35°C) on their biological characteristics.

2. MATERIALS AND METHODS

2.1. Insect cultures

Trichogramma cacoeciae and *T. bourarachae* used in this study were obtained from laboratory reared colonies initially collected from various locations in the oases of the South West of Tunisia and maintained in the laboratory of entomology of the Technical Center of Dates (TCD) at 25±1°C, 70±5% RH and a 14:10 (L:D) photoperiod. Parasitoids were reared on eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) as factious host which was maintained on semolina under the same climatic conditions used for the wasp. Eggs of *E. kuehniella* were glued on carton cards (1 x 5 cm) using Arabic gum diluted to 30% and then offered to parasitoids.

Ectomyelois ceratoniae was reared on date fruits until pupation at 27°C and 14:10 h (L:D) photoperiod. After emergence, adults were fed with a 10% honey solution inside petri dishes (diameter: 5.5 cm) lined with filter papers having the same diameter on which eggs were laid. Eggs were then collected and used for the trials.

2.2. Assessment of host suitability

Host suitability was studied by assessing selected biological parameters of *T. cacoeciae* and *T. bourarachae* on *E. ceratoniae* eggs. The method used in this study was that adopted by Roriz et al. (2006) with slight modifications. Females (24 h old) of each considered *Trichogramma* species were individually isolated in glass tubes (6 cm in length x 1 cm in diameter) containing each a carton card with 20 eggs of *E. ceratoniae* and a drop of honey solution as food. Egg cards were exposed to *Trichogramma* parasitism for 24h at 25°C and 16:8 h (L:D) photoperiod. After 24h, *Trichogramma* females were removed from the glass tubes. Cards with parasitized eggs were incubated inside the glass tubes to allow the preimaginal development of the parasitoids. Thirty replicates were considered for each *Trichogramma* species and the following parameters were evaluated: (i) the number of parasitized eggs that turned black, (ii) the number of offspring per host egg, (iii) the emergence rates and (iv) the sex ratio of the offspring.

To assess whether the development of parasitoids in *E. ceratoniae* eggs has an influence on the acceptability of this host by females of the (F1) generation, 30 females of *T. cacoeciae* and 30 females of *T. bourarachae* emerged from the eggs of *E. ceratoniae* were individually isolated in glass tubes (6 cm in length x 1 cm in diameter) and allowed to parasitize 20 eggs of this pest. *Trichogramma* females were kept for 24 h with host eggs at the same conditions described above before being removed from the glass tubes.

2.3. Biological traits of *T. cacoeciae* and *T. bourarachae* reared in *Ectomyelois ceratoniae* eggs

Females of *T. cacoeciae* and *T. bourrarachae* used in this trial emerged from eggs of *E. ceratoniae*. Thirty females of each *Trichogramma* species (24 h old) were individually isolated in glass tubes (6 cm in length x 1 cm in diameter) containing a carton card with 500 eggs of *E. kuehniella* commonly used for the rearing and the mass production of *Trichogramma* species. Each glass tube contained a drop of 10% honey solution as carbohydrate source. Tests were done at 25°C temperature and 16:8 (L:D) photoperiod. The egg cards were daily replaced by fresh ones during 7 days, and parasitized eggs were allowed to develop at the same conditions of their parents. After the 7th day, emerged wasps were maintained in glass tubes containing each a drop of 10% honey solution without egg cards until their death. The same experimental protocol was applied on females of the two *Trichogramma* species emerged from *E. kuehniella* eggs and the following parameters were evaluated: (i) number of parasitized host eggs that turned black during 7 days, (ii) emergence rates, (iii) sex ratio and (iv) longevity of females.

2.4. Effects of five different temperatures regimes on the biological traits of *T. cacoeciae* and *T. bourarachae*

Temperature regimes used in this study were 15, 20, 25, 30 and 35°C. The experimental protocol used was that adopted by (Ayvaz et al., 2008) with slight modifications. For each temperature, thirty females (24 h old) of each *Trichogramma* species were used to assess the parasitism rate and the mortality during 7 days. For each species, females parasitoid were individually isolated in glass vials (6 cm in length x 1 cm in diameter) containing a carton card with 50 eggs of *E. kuehniella* and a drop of 10% honey solution. The egg cards were daily replaced by fresh ones during 7 days and parasitized eggs were incubated at 25°C temperature and 16:8 h (L:D) photoperiod. The number of parasitized eggs and the number of dead females during the first 7 days were determined.

The impact of temperature on the development of parasitoids in their host was studied as described by (Young-Kyu et al., 2000 ; Bueno et al., 2009) with slight modifications. For both *Trichogramma* species, groups of 10 females (24h old) were placed in glass vials (6 cm in length x 1 cm in diameter), each containing a carton card with 30 eggs of *E. kuehniella*. Glass

Table 1. Suitability of eggs of *Ectomyelois ceratoniae* for *Trichogramma bourarachae* and *Trichogramma cacoeciae.*

	T. bourarachae		*T. cacoeciae*	
Experiment	**Parental generation**	**(F1) generation**	**Parental generation**	**(F1) generation**
Number of parasitized eggs	15,46 a (± 2,04)	16,00 b (± 2,90)	13,33 c (± 2,23)	14,33 d (± 2,13)
Emergence rate (%)	95,78 a (± 5,10)	94,78 a (± 5,58)	92,61 b (± 4,95)	90,41 b (± 4,97)
Number of offspring per parasitized egg	1,24 a (± 0,14)	1,27 a (± 0,12)	1,22 a (± 0,09)	1,25 a (± 0,21)
Sex ratio (% of females)	59,51 a (± 5,58)	58,84 a (±7,06)	100 b	100 b

Values are means ± SD. Means within one row followed by different letters are significantly different ($p < 0.05$).

Table 2. Influence of the development in the eggs of *Ectomyelois ceratoniae* on the biological characteristics of *Trichogramma bourarachae* and *Trichogramma cacoeciae.*

²	Experiment	*E. ceratoniae*	*E. kuehniella*
T. bourarachae	Number of parasitized eggs	79,10 a (± 20,62)	72,96 a (± 14,28)
	Emergence rate (%)	91,88 a (± 1,61)	91,17 a (± 2,57)
	Longevity (days)	15,60 a (± 4,63)	14,83 a (± 4,17)
T. cacoeciae	Number of parasitized eggs	57,8 a (± 13,63)	59,23 a (±10,59)
	Emergence rate (%)	89,82 a (± 3,17)	90,00 a (± 3,70)
	Longevity (days)	16,06 a (± 5,39)	17,10 a (± 5,88)

Values are means ± SD. Means within one row followed by different letters are significantly different ($p < 0.05$).

vials were then kept under controlled environmental conditions at 25±°C temperature and 16:8 h (L:D) photoperiod. The parasitism was allowed for 24h before removing parasitoids. Vials containing parasitized eggs were then placed in incubators set at different temperatures (15, 20, 25, 30 and 35°C) and 16:10 h (L:D) photoperiod. Five replicates were made for each *Trichogramma* species and each temperature regime. The effect of temperature on parasitoid development was estimated by measuring: (i) the mean developmental time of parasitoids (from egg to adult), (ii) the emergence rate of adults and (iii) the sex ratio.

2.5. Statistical 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 *Trichogramma* species (*T. cacoeciae* vs *T. bourarachae* and the generation (parental vs F1) on the performance of the wasps was analyzed using one way ANOVA followed by Duncan *post hoc* test.

In order to assess the impact of host species on the performance of *T. cacoeciae* and *T. bourarachae*, Student *t* test was used.

To assess the effects of temperature regime on the performances of *T. cacoeciae* and *T. bourarachae*, a factorial ANOVA was used. Subsequently, additional one-way ANOVA, followed by Duncan *post hoc* test for multiple comparisons were carried out. All statistical analyses were performed using R project for statistical computing (R Core Team, 2019).

3. RESULTS

3.1. Host suitability

Eggs of *E. ceratoniae* were more suitable for the development of *T. bourarachae* than that of *T. cacoeciae*. The mean number of parasitized eggs that turned black with *T. bourrarachae* prepupae (15.46±2.04) was significantly higher than that of *T. cacoeciae* (13.33±2.23). Similar results were obtained for the emergence of parasitoids from *E. ceratoniae* eggs, with the highest emergence rate recorded in the case of *T. bourarachae* (95.78%). The sex ratio and the mean number of offspring per host egg were similar for the two *Trichogramma* species (Table 1).

The development of *T. bourarachae* and *T. cacoeciae* in eggs of *E. ceratoniae* has not affected the acceptability of this host by females of the (F1) generation; in fact, the mean numbers of parasitized eggs of *E. ceratoniae* were similar in the parental generation (emerged from the eggs of *E. kuehniella*) and in the (F1) generation (Table 1). This test did not reveal significant differences between the (F1) generation and the parental generation for the emergence rates and the numbers of offspring per host egg.

3.2. Biological characteristics of *T. cacoeciae* and *T. bourarachae* reared on *E. ceratoniae* eggs

When *T. cacoeciae* and *T. bourarachae* were reared on eggs of *E. ceratoniae*, the number of parasitized eggs, the longevity period and the emergence rate of adults were similar to when *E. kuehniella* eggs were used as rearing host (Table 2).

3.3. Effects of five different temperatures on the biological characteristics of *T. cacoeciae* and *T. bourarachae*

The parasitism rate is significantly increased with increase in temperature from 15 to 30°C (Fig. 1.). For both *Trichogramma* species, the highest parasitism rate was obtained at 30°C and the mean numbers of host eggs parasitized at this temperature were 78.86 ± 12.87 and 59.66 ± 7.72, respectively for *T. cacoeciae* and *T. bourarachae*. The peak of parasitism of both *Trichogramma* species was recorded at the first 24 h of parasitism. *T. bourarachae* showed a better resistance to high temperatures; in fact, the mean numbers of eggs parasitized by females of *T. bourarachae* at 30 and 35°C were significantly higher as compared to *T. cacoeciae*. In addition, the longevity of *T. bourarachae* females (4.03 ± 0.56) at 35C° was significantly higher than that of *T. cacoeciae* (2.44 b ± 0.57) (Fig. 2.).

Trichogramma cacoeciae and *T. bourarachae* developed successfully from egg to adult at all tested temperatures except at 35°C (no adult emergence was observed at this temperature). For both *Trichogramma* species, the developmental time was significantly affected by temperature and it was shorter at the higher temperatures of the range (Table 3). The developmental periods were 29.18, 15.23, 12.00 and 10.44 for *T. cacoeciae* and 30.33, 15.72, 11.80 and 9.80 for *T. bourarachae* at the temperatures 15, 20, 25 and 30°C, respectively. The emergence rates were similar and relatively high at all the tested temperatures, ranging from 80,48 % to 97,92 % for *T. bourarachae* and from 92,03 % to 99,33 % for *T. cacoeciae* (Table 3). The overall sex ratio of emerged adults was female biased at all four temperatures, being 100% for *T. cacoeciae* and ranging from 66.52% to 70.48% for *T. bourarachae*. For both

Trichogramma species, there were no significant differences between the numbers of dead females during the first 7 days at all temperatures.

Table 3 Influence of the rearing temperature on emergence rate, developmental period and sex ratio of *Trichogramma bourarachae* and *Trichogramma cacoeciae*.

		Temperature (°C)			
		15	**20**	**25**	**30**
Emergence rate (%)	*T. bourarachae*	80,48 A a (± 15,18)	97,19 B a (± 2,84)	97,92 B a (± 3,02)	88,76 AB a (± 4,73)
	T. cacoeciae	97,53 A b (± 3,71)	99,33 A a (± 1,49)	94,63 A a (± 4,10)	92,03 A a (± 11,53)
Developmental period (day)	*T. bourarachae*	30,33 A a (± 1,08)	15,72 B a (± 1,31)	11,8 C a (± 0,77)	9,8 D a (± 0,77)
	T. cacoeciae	29,18 A b (± 1,04)	15,23 B a (± 1,03)	12,00 C a (± 0,84)	10,44 D b (± 0,52)
Sex ratio (% of females)	*T. bourarachae*	68,13 a (± 8,42)	68,21 a (± 18,23)	70,48 a (± 15,29)	66,52 a (± 15,13)
	T. cacoeciae	100 b	100 b	100 b	100 b

Values are means ± SD. Values within one row followed by different capital letters are significantly different ($p < 0.05$); data within one column followed by different lower-case letters are significantly different ($p < 0.05$).

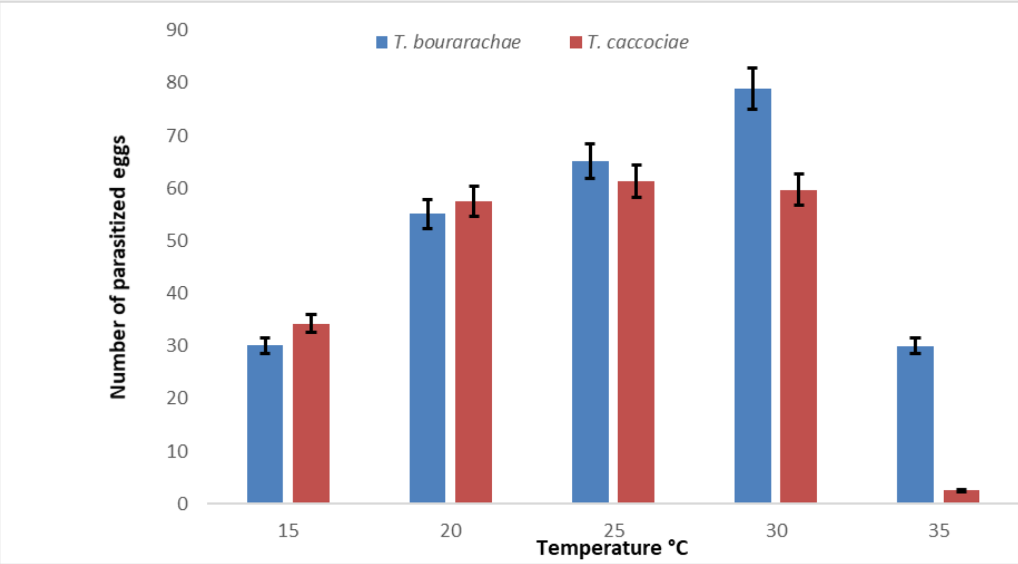

Fig 1. Influence of the rearing temperature on the number of host eggs parasitized by females of *Trichogramma bourarachae* and *Trichogramma cacoeciae*.

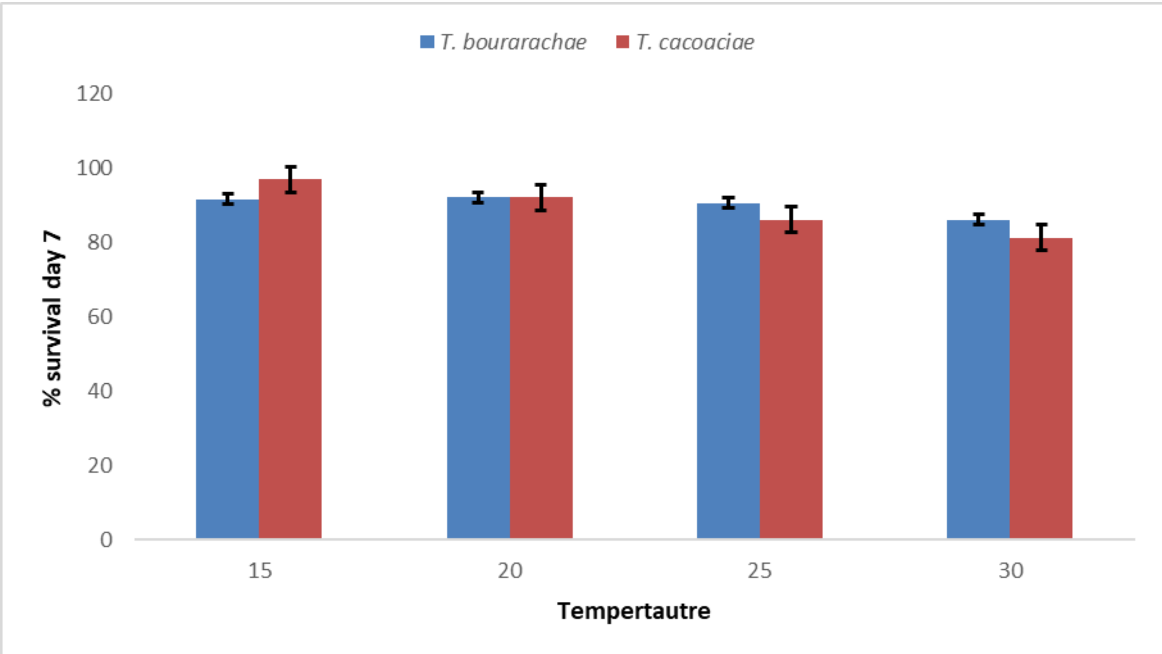

Fig 2. Influence of the rearing temperature on the percentage of females of *Trichogramma bourarachae* and *Trichogramma cacoeciae* who remain alive until day seven of the experiment.

4. Discussion

Our results showed that the host suitability differed among the *Trichogramma* species. Eggs of *E. ceratoniae* were more suitable for the development of *T. bourarachae*; in fact, the number of parasitized eggs as well as the emergence rate of adults was significantly higher as compared to *T. cacoeciae*.

Nurindach et al. (1999) found that female parasitoid usually accepts host eggs with about the same size as their natal host or larger ones. According to Brotodjojo & Walter (2006), the size of the rearing host eggs seems to affect the size and the biological characteristics of *Trichogramma* wasps. Liu et al. (1998) revealed that *Trichogramma* reared on smaller hosts are less robust and have lower performance than those reared on larger host eggs. In this work, wasps of *T. bourarachae* and *T. cacoeciae* developed in eggs of *E. ceratoniae* (0.5-0.8 mm long) (Balachowsky, 1972 ; Dhouibi, 1989) were similar in size to wasps emerged from *E. kuehniella* eggs (0.5 - 0.55 mm long) (Moreno et al., 1994), which may explain why the longevity of parasitoid females as well as the number of parasitized eggs were similar for *Trichogramma* wasps reared in the eggs of these two hosts. The number of individuals produced in a given host egg varied with the size of the host egg (Shirazi, 2006). Our results showed that, for both *Trichogramma* species, there was no significant difference between the mean numbers of offspring emerged from parasitized eggs of *E. ceratoniae* and *E. kuehniella*, this can be due to the similar size of the eggs of these two hosts.

The biological characteristics of *T. bourarachae* and *T. cacoeciae* were influenced by temperature under laboratory conditions, indicating that this parameter might affect the success of biological control programs using these two *Trichogramma* species. Temperature directly affected the developmental period of *T. bourarachae* and *T. cacoeciae*. According to Cossins & Bowler (1987), almost in all organisms there is a positive acceleration in the biological parameters when temperature increases within a thermal range, which is probably a consequence of the increase in the metabolic activity of the parasitoid (Bueno et al., 2009). In this work, the shortening of parasitoids developmental time (from egg to adult) inversely follows the increases in the temperature. Similar results were reported by (Young-Kyu et al., 2000) who found that the rate of development of *T. dendrolimi* accelerated as temperature increased. The same think was revealed by Scholler & Hassan (2001) for the species *T. evanescens*, the developmental time decreased from 9 days at 26°C to 7 days at 30 °C.

Many studies showed no consist relationship between temperature and sex ratio of *Trichogramma* species like in the case of *T. minutum* (Lund, 1934) and *T. pretiosum* (Calvin et al., 1984). In this work, temperature did not affect the sex ratio of *T. bourarachae* and *T. cacoeciae* and the percentage of females that emerge from parasitized eggs of *E. kuehniella* was always higher than that of males. However, Harrison et al. (1985) reported that the temperature is capable of causing changes in the sex ratio of *T. exiguum* and *T. pretiosum*. Young-Kyu et al. (2000) and (Lund, 1938) revealed also that temperature could affect the sex ratio of *T. dendrolimi* and *T. evanescens*.

The emergence rate of *T. bourarachae* and *T. cacoeciae* from parasitized host eggs was not affected by temperature. Similar results were found by Harrison et al. (1985) for *T. exiguum* and *T. pretiosum*. However, Young-Kyu et al. (2000) reported different results from ours, temperature significantly influenced *T. dendrolimi* emergence rate, which tended to be lower at the higher temperatures.

In this work, the highest numbers of parasitized eggs were obtained at 30°C. Shirazi (2006) has also found a significantly better performance of *T. chilonis* (> 85 eggs/female/life time) at 30°C. Although parasitization by *T. bourarachae* and *T. cacoeciae* was observed at 35 °C, no adults emerged from the blackened eggs at this temperature. Similar results were obtained by Scholler & Hassan (2001) who found that the eggs parasitized by *T. evanescens* turned black at 35°C but no adult emergence was observed at this temperature. According to Ayvaz et al. (2008), the fact that parasitized eggs are killed, regardless of parasitoid emergence, suggests that *Trichogramma* species could be a desirable candidate for mass rearing and release as a biological control agent. Due to the tolerance of *T. cacoeciae* and *T.bourarachae* to high temperatures, these two species could be candidates for use in the control of thermophile pyralid moths like *E. ceratoniae*.

Despite that the number of parasitized eggs varied among the different temperatures, the oviposition peak of *T. cacoeciae* and *T.bourarachae* was always at the first 24 h of parasitism. Many studies have revealed that the oviposition peak of egg parasitoids from the genus *Trichogramma* was usually at the first day after adult emergence (Bai et al., 1992 ; Volkoff & Daumal, 1994 ; Bueno et al., 2012). Mills & Kuhlmann (2000) explain this by the fact that most of *Trichogramma* species have the capacity to store a full complement of mature eggs in the ovaries or oviducts and complete oogenesis either before or shortly after adult emergence (pro-ovigenic parasitoids) and, thus, adults emerge ready to lay eggs.

Many studies have reported that the longevity of different *Trichogramma* species and strains was decreased as a consequence of the temperature increase (Hansen & Jensen, 2002; Maceda et al., 2003; Bueno et al., 2012). Due to the pro-ovigenic behavior of *Trichogramma* species, we followed in this work the mortality of females of *T. cacoeciae* and *T.bourarachae* during the first 7 days after adult emergence. For both *Trichogramma* species, there were no significant differences between the numbers of dead females at temperatures between 15 and 30°C. Although females of *T. cacoeciae* and *T. bourarachae* didn't live until the 7th day at 35°C, the longevity of *T. bourarachae* females (4.03 ± 0.56) at this temperature was significantly higher as compared to that of *T. cacoeciae* (2.44 ± 0.57).

5. CONCLUSION

Eggs of *E. ceratoniae* were more suitable for the development of *T. bourarachae* than that of *T. cacoeciae*. The development of *T. bourarachae* and *T. cacoeciae* in eggs of *E. ceratoniae* has not affected the acceptability of this host by females of the (F1) generation. The parasitism rate significantly increased with increase in temperature from 15 to 30°C. For both *Trichogramma* species, the highest parasitism rate was obtained at 30°C. The developmental time of *T. bourarachae* and *T. cacoeciae* was significantly affected by temperature and it was shorter at the higher temperatures of the range. *Trichogramma bourarachae* showed a better resistance to high temperatures than *T. cacoeciae*.

Overall, *Trichogramma* species would obviously enhance the control performance against *E. ceratoniae* in Tunisia. Many experiments should be applied in future to reach the adequate trials including density of *Trichogramma* release for an Integrated Pest Management (IPM) against this pest in Tunisian oases.

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