Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2022; 10(2): 28-35

Received: 04-02-2022
Accepted: 14-03-2022

Anis Zouba
Technical Centre of Dates, 4280 Kebili, Tunisia

Sahar Zougari
1. Technical Centre of Dates, Kebili, Tunisia
2. Department of Biological Sciences, Faculty of Science of Tunis. University of Tunis El Manar, 20 street of Tolède Tunis, Tunisia
3. Department of Plant Health and Environment. LR14AGR02 of Bioaggressors and Integrated Pest Management in Agriculture, National Institute of Tunisia. University of Carthage Street Charles Nicolle, Tunis, Tunisia

Sabrine Attia
Department of Plant Health and Environment. LR14AGR02 of Bioaggressors and Integrated Pest Management in Agriculture, National Institute of Tunisia. University of Carthage 43 street Charles Nicolle Tunis, Tunisia

Khaled Abbes
High Agronomic Institute of Chott-Mariem, University of Sousse, Chott-Mariem, Sousse, Tunisia

Kaouthar Grissa Lebdi
Department of Plant Health and Environment. LR14AGR02 of Bioaggressors and Integrated Pest Management in Agriculture, National Institute of Tunisia. University of Carthage 43 street Charles Nicolle 1082 Tunis, Tunisia

Brahim Chermiti
High Agronomic Institute of Chott-Mariem, University of Sousse, Chott-Mariem, Sousse, Tunisia

Foued ben Hmida
Technical Centre of Dates, Kebili, Tunisia

Corresponding Author:
Sahar Zougari
1. Technical Centre of Dates, Kebili, Tunisia
2. Department of Biological Sciences, Faculty of Science of Tunis. University of Tunis El Manar, 20 street of Tolède Tunis, Tunisia
3. Department of Plant Health and Environment. LR14AGR02 of Bioaggressors and Integrated Pest Management in Agriculture, National Institute of Tunisia. University of Carthage Street Charles Nicolle, Tunis, Tunisia

Field performance of the egg parasitoid *Trichogramma cacoeciae* Marchal (hymenoptera: Trichogrammatidae) following releases against *Ectomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae) in two types of oasis in Tunisia

Anis Zouba, Sahar Zougari, Sabrine Attia, Khaled Abbes, Kaouthar Grissa Lebdi, Brahim Chermiti and Foued ben Hmida

DOI: https://doi.org/10.22271/j.ento.2022.v10.i2a.8967

Abstract
This study was carried out in two oases located in the south-west of Tunisia to investigate the efficacy of the oophagous parasitoid *Trichogramma cacoeciae* against *Ectomyelois ceratoniae* on date palm trees. The first oasis (Hezoua) is characterized by a cropping system divided into three levels of cultures. In the second oasis (Douz), orchards were based on the monoculture of the Deglet Noor cultivar. Parasitoids were released with three doses 26,000, 36,000 and 46,000 Trichogramma/ha. The impact of *Trichogramma* releases was evaluated by monitoring the egg parasitism, male catches of *E. ceratoniae* through pheromone traps, fruit damage during the whole season and fruit infestation at harvest. *Trichogramma cacoeciae* accomplished higher egg parasitism in Hezoua oasis (19.71 ± 1.02%) as compared to Douz oasis (8.43± 1.03%). Inundative releases of *T. cacoeciae* in the two oases resulted in varying degrees of pest control and they were not sufficient to limit economic losses.

Keywords: Biological control, inundative release, egg parasitism, Trichogramma, *ectomyelois ceratoniae*, date palm cultivation

1. Introduction
Date palm (*Phoenix dactylifera* L.) is one of the most important fruit crops in Tunisia, having a key role in the stability and continuity of life of local populations. Tunisian dates are exported to more than 80 countries around the world and the Deglet Noor variety accounts for the majority of date production. The date palm in southern Tunisia is often prone to several phytosanitary problems, mainly pests which are considered to be a major threat to the date sector [1]. In particular, the carob moth *Ectomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae) is a cosmopolitan insect pest of crops of economic importance in many parts of the world [2]. In Tunisia, this highly polyphagous insect is the most destructive pest attacking dates in the field and storage facilities [3]. Indeed, date infestation increases the risk of compromising exports particularly with the cultivar Deglet Noor which is one of the most affected varieties with losses that can reach 20% [4].
There is now an urgent need for the implementation of effective control methods to keep carob moth populations below economic thresholds and satisfy international regulations and standards necessary for export [5]. Chemical control seems to be not satisfactory on dates and pomegranates due to the endophytic behaviour of *E. ceratoniae* [6]. Various control techniques were tested to substitute the use of chemical insecticides and strengthen integrated pest management (IPM) against *E. ceratoniae* such as autocidal control [5], biotechnical control by mass trapping (based on pheromone traps) [7] and mating disruption [8], and biological control by the release of egg and larval parasitoids [3, 9].
Wasps of the genus *Trichogramma* oviposit predominantly in the eggs of a large number of pests and they eliminate them even before they began to cause damage [10]. In fact, Trichogrammatids are the most widely used parasitoids in the control of agricultural pests, particularly against Lepidopterans [11].

In Tunisia, several native species of *Trichogramma* have been tested for the biocontrol of *E. ceratoniae* [12]. In particular, *Trichogramma cacoeciae* (Marchal) is a thelytokous parasitoid with a broad geographic distribution [13]. A native strain of *T. cacoeciae*, selected by Khoualdia *et al*. (1995) [14], is currently used in IPM program against *E. ceratoniae* in pomegranate orchards in the south-east and the north of Tunisia.

The present study allowed us to estimate the field performance of *T. cacoeciae* through inundative releases in two types of oasis during the fruiting season of 2015 and to outline prospects for practical pest management of the carob moth by this environmentally friendly and sustainable control technique.

2. Material and Methods

2.1. Experimental plots

The study was conducted during 2015 in two oases located in the south-west of Tunisia: (1) Hezoua oasis belonging to the governorate of Tozeur and (2) Douz oasis belonging to the governorate of Kebili. For both oases, date palm trees were planted at 8 m within and between rows, totalling around 150 trees/ha. In Hezoua oasis, the experimental orchard is characterized by a cropping system divided into three levels of cultures: date palm trees of the Deglet Noor variety in the upper level, fruit trees (*Ficus carica* L.,, *Citrus sinensis* L., *Punica granatum* L.,) in the second level, vegetable (*Solanum lycopersicum*, L., *Capsicum annuum* L.) and fodder crop (*Medicago sativa* L.) in the third level. In Douz oasis, the experimental orchard is based on the monoculture of the Deglet Noor cultivar. In each oasis, a 11.5 ha experimental orchard was partitioned into three replicated blocks of approximately 3.5 ha each. Each block was divided into four 0.5 ha plots, one for release of each of the three doses of *T. cacoeciae* (D1: 26 000 T/ha, D2: 36 000 T/ha, D3: 46 000 T/ha) and one for the control. Plots were separated from each other by separation areas of 50 m large to avoid interference between tested doses. Date palm orchards in Hezoua and Douz oases were irrigated by the flood system and the drip-irrigated system, respectively.

2.2. Climatic conditions

The experimental orchards were equipped with climatic data loggers (WatchDog A-Series) to monitor changes in temperature and relative humidity throughout the period of study. Precipitations were recorded from two weather stations located in Tozeur and Kebili.

2.3. Monitoring of *Ectomyelois ceratoniae*

The flight activity of *E. ceratoniae* males was monitored by placing one delta pheromone trap (ISCA® Technologies) per plot (0.5ha) at a height of 1.5 m on date palm trees to allow proper timing of *Trichogramma* releases. Traps were installed on 04 and 10 September 2015 in all experimental plots in Hezoua and Douz orchards, respectively. Pheromone traps were also installed in the center of each of the control plots. Traps were checked weekly for captures of males. Pheromone lures (ISCA lure *E. ceratoniae*) and sticky cards were renewed every month during all the study period.

2.4. Origin and mass rearing of *Trichogramma caoeciae*

The indigenous strain of *T. cacoeciae* used in this study was obtained from a laboratory colony, initially started with individuals isolated from eggs of *E. ceratoniae* in Tozeur oases and identified using molecular methods [15]. Parasitoids were reared on fresh eggs of the factitious host *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), which was maintained at 25±1°C, 70±5% RH and a 16:8 (L:D) photoperiod [16]. Ultraviolet-irradiated eggs of *E. kuehniella* were glued on cardboard cards (1 x 5 cm) using arabic gum (30%) and then offered to parasitoids in culture glass tubes (1 x 10cm) plugged with cotton. Glass tubes were placed in the climatic room under the same climatic conditions used for the host. The obtained parasitized eggs were transferred into new glass tubes until hatching. A drop of honey was placed in the tube to feed the new emmerged wasps, which were provided with newly laid *E. kuehniella* eggs.

2.5. Field releases of *Trichogramma cacoeciae*

Two inundative releases of *T. cacoeciae* took place from 11 September to 02 November 2015 at two weeks intervals to cover the occurrence of the two generations of *E. ceratoniae* on date palm trees. Cardboard cards (1 x 5 cm) containing each 1000 *E. kuehniella* eggs parasitized by *T. cacoeciae* were used as releasing units in this study. Eggs contained parasitoids of different developmental stages to ensure a continous emergence and presence of the wasp in the treated fields. As ants were proven to be predominantly responsible for the removal of considerable amounts of parasitized eggs and sentinel eggs in the field [15, 17], each cardboard card was protected with a release boxe (5 cm × 3 cm × 2 cm) covered with 0.75 mm mesh screen to allow *Trichogramma* emergence and prevent any predators from entering. Date palm trees used for *Trichogramma* releases were selected folowing a zigzag pattern. Release boxes were installed on date bunches in the southern direction of palm trees.

2.6. Assessment of the efficacy of *Trichogramma cacoeciae* releases

2.6.1 Emergence rate of released *Trichogramma cacoeciae*

In order to control the emergence rate of *T. cacoeciae*, ten cardboards were taken randomly from each experimental plot seven days after each release and examined under a binocular microscope (Leica® model MS5). Hatched and unhatched eggs were counted to compare the emergence rate between open field conditions and laboratory conditions. The emergence rate was calculated as follows:

Emergence rate = (Number of eggs with emerged parasitoids/ Total number of eggs) x 100

2.6.2 Evaluation of *Trichogramma cacoeciae* parasitism rate in the field

Parasitism was assessed by checking the presence of *E. ceratoniae* eggs in fruit samples (200 date fruits/ plot/ week). Date fruits were collected randomly from the four cardinal orientations of 10 date palm trees. Eggs of the target pest were collected from sampled fruits, placed on cardboard cards (1 cm × 4 cm) and kept in a climatic chamber (25°C, about 70% RH, 16:8h L:D). Eggs were examined daily under a binocular microscope and those hatching into larvae were recorded. Parasitized eggs were easily identified by their black color and their number was recorded. In a separate experiment, the parasitism rate was determined under laboratory conditions using cardboard cards containing 100 fresh eggs of *E. ceratoniae*.

The parasitism rate was calculated as follows

Parasitism rate = (Total number of parasitized eggs of *E. ceratoniae*/ total number of eggs) x 100

2.6.3 Evaluation of field infestation by *Ectomyelois ceratoniae*

The impact of *T. cacoeciae* releases on *E. ceratoniae* density and damage was determined by the following parameters in control and treated plots: (1) egg and larval density of the target pest, (2) fruit damage during the entire season and (3) fruit infestation at harvest. A sample of 200 date fruits/week/plot was collected randomly from 10 date palm trees from 22 September until 02 November 2015. Date fruits were collected from the four cardinal orientations of the tree. At harvest time, 5 palm trees/plot were selected randomly and the infestation rate of fruit harvest/ tree was determined. The infestation rate was calculated according to the following formula:

Infestation rate = (NA/NB) *100.

Where:
NA: the number of infested fruits, NB: the total number of fruits The reduction rate was calculated according to Abbott (1925) formula as follows:

Reduction rate = ((A-B/A) *100).

Where:
A: the mean infestation of date fruits/ week in the control treatment,
B: the mean infestation of date fruits/ week in the treated plot.

2.7. Statistical analyses

IBM SPSS Statistics 20 version was used to perform all statistical analysis. The obtained data corresponding to the emergence rate, parasitism rate, and infestation rate caused by *E. ceratoniae*. Additional one-way ANOVA followed by Tukey *post hoc* tests at p = 0.05 inside each treatment was carried out after checking the normality test of all data sets.

3. Results

3.1. Emergence rate

On average, the numbers of emerged adults in the Hezoua oasis during the two releases were 835, 896 and 817 *Trichogramma*/1000 parasitized eggs respectively for D1: 26.000, D2: 36.000 and D3: 46.000; corresponding to emergence rates of 83.5%, 89.6% and 81.7%, respectively. For all treatments, the emergence rate did not vary significantly in the field compared to control (under laboratory conditions) (F (9.23, 4.25) = 10.03; p= 0.993). As shown in table 1, the emergence rates recorded after the first and the second release was similar and relatively high, ranging from 81.46% to 84.93%.
In Douz oasis, the emergence rate was significantly higher in laboratory conditions compared to first and second releases for the three doses of *T. cacoeciae* (F(6.14, 3.21)= 9.87; p=0.002) (Table 1). The emergence rates registered after the two releases of parasitoids were relatively low, varying from 34.2% to 48.6%.

Table 1: Emergence rate (± SE) of *T. cacoeciae* adults from parasitized eggs of *E. kuehniella* in experimental orchards and under laboratory conditions

	Emergence rate (%) in laboratory	Emergence rate (%) in Hezoua orchard	Emergence rate (%) in Douz orchard
Release 1	96.7 ± 1.03Aa	84.93± 2.76Aa	34.2 ± 3.46aB
Release 2	93.5 ± 1.98Aa	81.46 ± 3.54Aa	48.6 ± 5.66aB

*Numbers followed by the same letter(s) within a row (lower case letter) and within a column (upper case letter) are not significantly different at 5%.

3.2. Parasitism rate

In Hezoua oasis, the first inundative release was performed at the beginning of the oviposition period of the first generation of *E. ceratoniae*, when the average numbers of eggs on the sampled fruits were 3, 1 and 5 eggs/ 200 dates, respectively in plots treated with doses D1, D2 and D3. After the first release, parasitism rate varied from 5 to 11% and it increased over time to reach its maximum during the 2nd release with 34%, 36% and 41% for D1, D2 and D3, respectively (Figure. 1a). No significant differences were detected between D1, D2 and D3 in parasitizing E. ceratoniae eggs. The results in table 2 showed a significant difference in the parasitism rates between laboratory and plots treated with the three doses (F (7.54, 3.64) = 13.54; p=0.887).

Table 2: Parasitism rate (± SE) of *T. cacoeciae* on *E. ceratoniae* eggs in laboratory and field conditions.

	Laboratory	D1	D2	D3
Hezoua	81.43 ±3.45 a	19.71 ± 1.02b	21.43 ±2.87b	24.71 ± 3.76b
Douz	81.43±3.45a	8.43± 1.03b	9.28±0.97b	10±1.12b

*Numbers followed by the same letter(s) are not significantly different at 5%.

In Douz oasis, the first release was conducted in the beginning of the infestation, when the average numbers of eggs on the sampled fruits were 1, 2 and 1 eggs/ 200 dates, respectively for D1, D2 and D3 treated plots. One week after the 2nd release, the maximum parasitism rate in this oasis was 16% for D1 compared to 18% for D2 and 16% for D3 (Figure. 1b). No significant differences were observed between the three tested releasing doses in terms of parasitism of the moth eggs (F (7.89, 8.56) = 11.23; p= 0.993). Furthermore, results showed a significant differences in the parasitism rates between laboratory and plots treated with D1, D2 and D3 (F (6.47, 5.14) = 8.76; p= 0.041). The highest parasitism rate was recorded at harvest in polts treated with 46.000 T/ha.

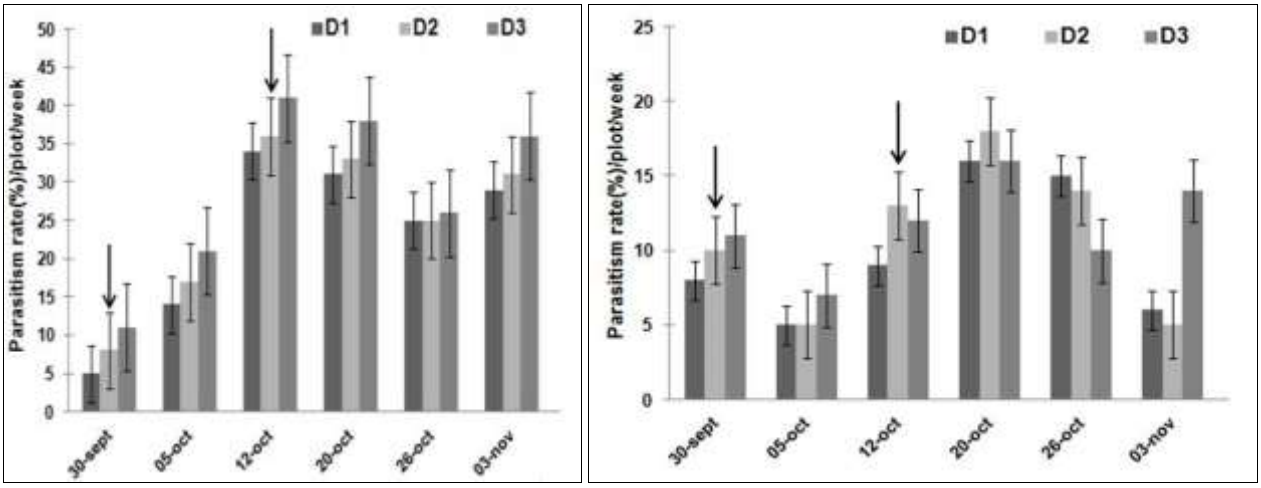

Fig 1: Weekly parasitism rate of *E. ceratoniae* eggs after releases of 26.000 (D1), 36.000 (D2) and 46,000 (D3) *Trichogramma*/ha on date palm trees in Hezoua (a) and Douz (b) orchards during 2015. (Arrows indicate the dates of releases for the three tested doses).

3.3. Male catches on date palm trees

In Hezoua oasis, results of male trapping through pheromone traps showed the presence of the carob moth in experimental plots during all the study period (Figure 2). For the first release of *T. cacoeciae*, we recorded 0; 1; 1 and 0.5 males/ trap for D1, D2, D3 and control plot, respectively. Fluctuation of *E. ceratoniae* males showed maximum catches during the second release of *Trichogramma* (12 octobre 2015) with 5; 4; 4 and 6 males/ trap for D1, D2, D3 and control, respectively. After *Trichogramma* releases, statistical analyses showed a significant difference between the treated plots and control in capturing carob moth males ($F(2.035, 27) = 12.46$; $p = 0.0031$). The pest density was reduced by 22.22% (D1), 30.55% (D2), and 33.33% (D3), respectively. Similar patterns in male catches for the three tested doses ($F(2.47, 16.53) = 7.83$; $p = 0.965$) were observed.

The flight activities of *E. ceratoniae* in Douz oasis is shown in Figure 2b. A major peak of the moth was registered after one week of the 2nd release of *T. cacoeciae* (21 octobre 2015) with 4; 4; 3 and 7 males/ trap for D1, D2, D3 and control, respectively. During this experiment, a total number of trapped males was 12; 12; 10 and 18 males/trap in D1, D2, D3 and untreated plot, respectively. A significant difference in the number of trapped males was detected between the treated plots and the control ($F(1.256, 14) = 9.87$; $p = 0.034$). The pest density was reduced by 30.55, 33.33 and 41.66% for D1, D2 and D3, respectively. However, the number of trapped males was similar in plots treated with the three release doses ($F(3.49, 16) = 7.89$; $p = 0.879$).

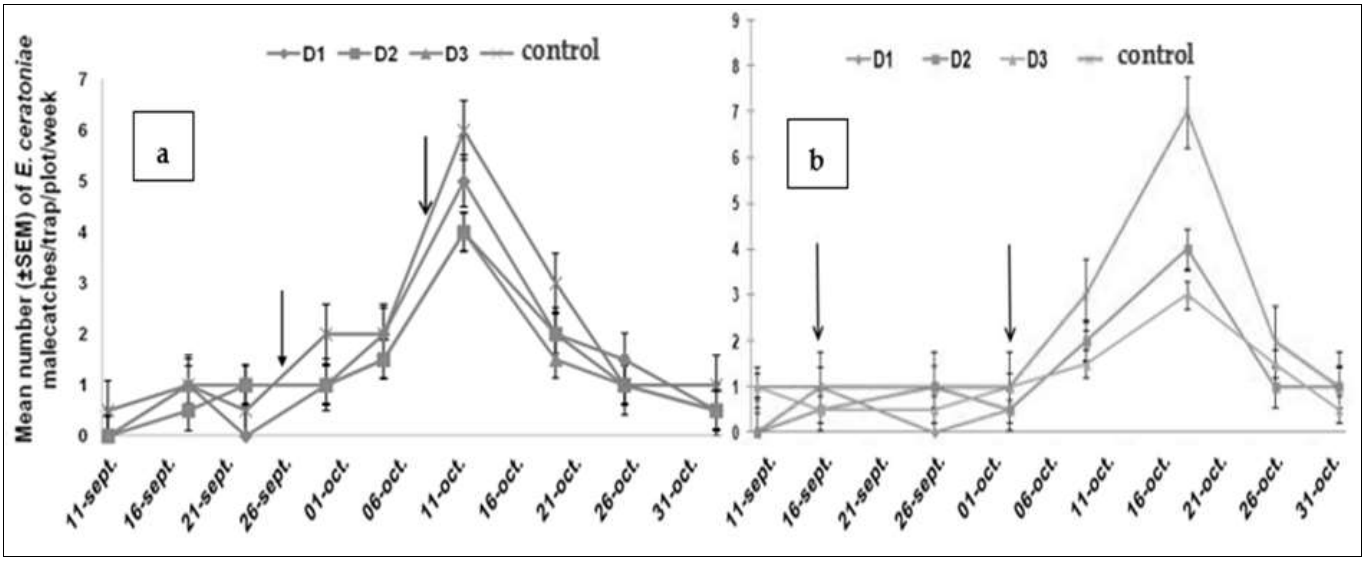

Fig 2: Weekly catches of *E. ceratoniae* males per Delta traps on date palm trees in Hezoua (a) and Douz (b) orchards during 2015. (Arrows indicate release's date of *Trichogramma* for the three tested doses)

3.4. Assessment of field infestation by *Ectomyelois ceratoniae*

In Hezoua oasis, the mean infestation rate of date fruits during the entire season is 7.5, 10 and 11% after treatment with D1, D2 and D3, respectively. No statistical differences were recorded between release doses ($F(1.27, 13) = 11.24$; $p = 0.188$). However, a significant reduction in the date fruit infestation was observed between treated and control plots ($F(3, 27) = 1.73$, $p = 0.003$). The highest infestation rates matched with the second generation of the carob moth (12 octobre 2015) with 22; 14 and 17% for D1, D2 and D3, respectively (Figure 3a). In Douz oasis, the infestation rate of fruits in control and treated plots increased gradually until the end of the season and it was more accentuated in October compared to September (Figure 3b). The mean infestation rate during the season was 15, 10 and 11% after releases of D1,

D2 and D3, respectively. No statistical differences were recorded between the three released doses (F (3.47, 11) = 9.23; p =0.995). Nevertheless, results showed a significant difference in the infestation rates between the control and the treated plots (F(3,27)=0.851, p = 0.048). At the harvest (November), the infestation rate of fruits in the Hezoua oasis was significantly higher in the control plots with 16.8% compared to 8.6, 8 and 7.8% after treatment with D1, D2 and D3, respectively (Figure 11). Therefore, we registered a reduction rate of 48.81, 52.38 and 53.57% for D1, D2 and D3, respectively. In Douz oasis, date fruits infestation was high at harvest with 35% for control plots and 25; 34 and 33% for D1, D2 and D3, respectively (Figure 4). The reduction rates registered in treated plots were 28.57; 2.85 and 5.71% for D1, D2 and D3, respectively.

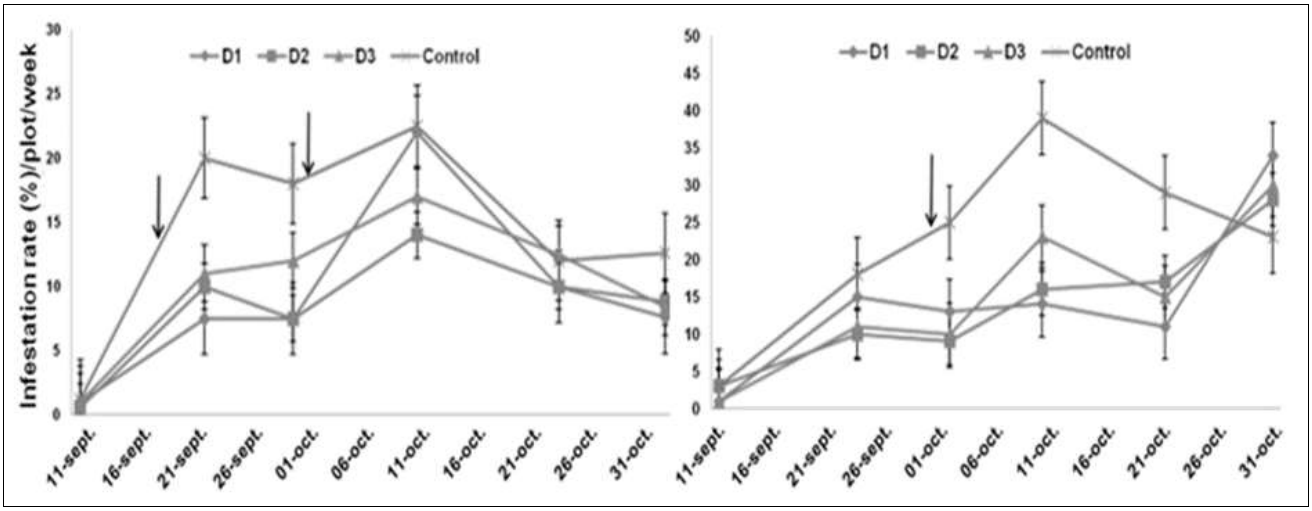

Fig 3: Weekly evolution of the infestation rate of fruits in Hezoua (a) and Douz (b) orchards during 2015. (Arrows indicate the release's date of *Trichogramma* for the three tested doses)

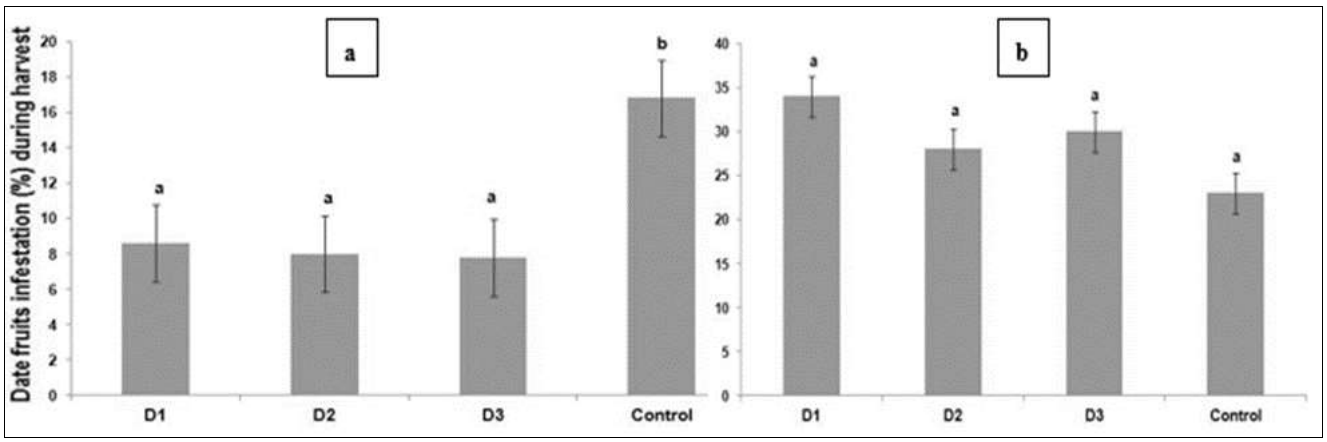

Fig 4: Infestation rates of date fruits at harvest in control and plots where *T. cacoeciae* was released using the doses D1, D2 and D3 in Hezoua (a) and Douz (b) orchards during 2015. [Bars bearing the same letter are not significantly different (p≤0.05; Tukey's test].

4. Discussion

In Hezoua oasis, the emergence rate in experimental plots was relatively high for the three tested doses (> 80%). However, in Douz oasis field conditions have significantly influenced parasitoid emergence from *E. kuehniella* eggs and the emergence rates were higher in the laboratory. This difference between field and laboratory conditions could be explained by several factors such as weather and predation [18]. In Douz oasis, we registered extreme tempertaures throughout the period of study and the maximum temperature recorded was 37.5°C which can affect the emergence rate of *T. cacoeciae*. Zouba *et al*. (2021) [19] showed that *T. cacoeciae* emerged successfully from host eggs at 15, 20, 25 and 30°C but not at 35°C. Similarly, Ghosh *et al*. (2017) [20] showed that *Trichogramma exiguum* Pinto & Platner emergence was significantly reduced in response to increasing temperatures of 35°C or higher. Experimental plots in Douz oasis are considered as areas of bright light intensity and of dry climate (22 - 37.5 °C, HR<50%) due to the monoculture of the Deglet noor variety, which may disrupt the wasps emergence. In Hezoua oasis, the high diversity and density of trees and herbaceous plants and the flood system's irrigation provide a favorable microclimate for *T. cacoeciae*. In fact, this oasis is charactirized by a moderate sunlight and we registred adequate temperature and relative humidity conditions (11- 31 °C, R.H ≥ 50%). We noticed in this study that predation reduced the number of parasitoids available for emergence in the field below those originally planned. Ants appeared to be the first responsible for this predation, since they were the only potential predators found inside the release boxes. Predation has been reported in other studies in which the encapsulated material was not used, with losses up to 50% in corn and 91-98% in cotton [21]. However, predation was not observed in cotton and apple orchards treated with encapsulated *T. exiguum* [22] and in corn treated with encapsulated *T. brassicae* [34].

Since *E. ceratoniae* is a multivoltine species, inundative releases of *Trichogramma* should be timed to coincide with the begening of the oviposition period of the pest [16]. In our study, the first release of *T. cacoeciae* in Hezoua and Douz oases was made when the average number of *E. ceratoniae* eggs was 3 and 1.33 eggs/ sample of 200 date fruits and the average number of captured males was 0.5 and 1 male/ delta trap, respectively.

Trichogramma cacoeciae showed higher performance in the laboratory compared to field conditions. This lower performance in the field can be due to the mass rearing conditions, weather, habitat, crop, host, and parasitoid quality. Several studies showed that mass rearing under variable temperatures and light regimes do not produce heat resistant parasitoids and can impact negatively the parasitism capacity of *Trichogramma* in natural field conditions [23]. Climatic factors such as temperature, humidity and wind were found to have a significant influence on the fecundity, longevity, host searching capacity and walking activity of *Trichogramma* wasps [24]. The habitat provides a variety of factors that may be limiting for *Trichogramma* augmentation programs [23]. Several studies have shown that the size of the plant, its surface area, its complexity and its leaf surfaces may affect the ability of *Trichogramma* wasps to disperse, search and locate hosts [24]. Our results demonstrated that the indigenous strain of *T. cacoeciae* was not very successful in searching and parasitizing eggs of *E. ceartoniae* on date palm trees under field conditions. In fact, the parasitism rate of the target pest eggs was relatively low in Hezoua and Douz oases with a maximum of 41% and 18%, respectively. Compared to previous studies in date palm orchards using *T. cacoeciae*, the parasitism of *T. cacoeciae* on *E. ceratoniae* eggs reached 18.3% and 78.7%, respectively [7, 9].

The monitoring of the flight activity of the carob moth in Douz and Hezoua oases showed the presence of the pest in date palm orchards during the entire season (September-November). The major peak of male catches registered in October corresponds to the last generation of the pest before overwintering and coincides with the dates ripening period. The highest number of moths was trapped in control plots with a total of 18 males/ delta trap in both oases. A significant reduction of male captures was observed between tested doses (D1, D2 and D3) and control.

The biological control of the carob moth *E. ceratoniae* in Douz and Hezoua oases resulted in varying degrees of pest control. In fact, the mean infestation rate of date fruits at harvest was significantly higher in Douz oasis (30.66%) compared to Hezoua oasis (8.13%). This difference may suggest that the parasitoid is better adapted to the second oasis, thanks to its favourable micro-climate and its flora diversity. Field releases of *T. cacoeciae* using the dose D1 (26,000 T/ha) seems to be suitable to reduce the impact of *E. ceratoniae* on date palm trees since there was no significant difference recorded for the three tested doses on date fruit losses. This dose has led to a significant reduction in fruit damage in comparison to control plots. However, the inundative release of *T. cacoeciae* in date palm orchards was not sufficient to limit economic losses mainly in Douz oasis.

Several studies evaluated the efficacy of *T. cacoeciae* releases to protect arboreal and horticultural crops. The actual rates of release vary considerably, even for the same pest and crop. In tomato greenhouses, releasing of 25,000 adults of either *T. cacoeciae* or *T. bourarachae* per plant induced a reduction of 78.89% and 87.62% of *Tuta absoluta* damages, respectively [15]. In the Tastour region, releases of 15.000 *T. cacoeciae* per pomegranate tree reduced fruit infestation from 13.2% in untreated plot to 2.4% in a treated plot [26]. Besides, Dhouibi *et al*. (2016) [27] showed a decrease in the infestation level from 82% to 22% by releasing 25.000 *T. cacoeciae* per hectare in pomegranate orchards. According to Khoualdia (2006) [28], the release of a total of 157.000 *T. cacoeciae*/ha on date palms reduced the infestation rate from 16.5% in the control to 3.5% in the treated plot. In citrus groves, the efficacy of *T. cacoeciae* releases (3000 wasps/tree/release) was about 44.5% at harvest [29].

Trichogramma wasps released in combination with other compatible control methods can enhance the control of targeted pests. Ben Chaabane and and Mahjoubi (2019) [30] evaluated the efficacy of *T. cacoeciae* releases combined with mass trapping on pomegranate and date palm trees. Several authors have studied the possiblity of using *Trichogramma* in combination with *Bacillus thuringiensis* (Bt) and *Beauveria bassiana* [31]. Other authors suggest that releases of more than one species of *Trichogramma* can improve efficacy in field conditions [32]. Some studies suggested to combine *Trichogramma* releases with other biological control agents such as parasitoids (*habrobracon hebetor* and *Telenomus remus*) [33], predators (*Chrysoperla carnea*) [34] and entomopathogenic nematodes (*Heterorhabditis bacteriophora*) [35].

5. Conclusion

In conclusion, the decrease of the infestation level in date palm orchards after two inundative releases of the indigenous strain of *T. cacoeciae* was not sufficient to limit economic losses suggesting that, this biological agent could be used as a part of an integrated pest management program of the carob moth *E. ceratoniae*. Further studies are needed to test *Trichogramma* releases in combination with other techniques of control such as mass trapping through pheromone traps and mating disruption technique.

6. Acknowledgments

The authors are very grateful to Mourad Lassoued, Tayeb Chatter and Mohamed Salah Legtari for their technical assistance.

7. References

1. Lebdi-Grissa K, Attia S, Sahraoui H, Cherif A, Hached W, Zougari S, *et al.* Les moyens alternatifs de lutte en agriculture biologique en Tunisie, cas de la pyrale des dattes, *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae) et de la mineuse de la tomate, *Tuta absoluta* (Lepidoptera: Gelechiidae). Revue des Régions Arides. 2020;46(1):331-344.
2. Nay JE. Impact of ant predation and heat on carob moth (Lepidoptera: Pyralidae) mortality in California date gardens. Journal of Economic Entomology. 2005;98:725-731.
3. Khoualdia O, R'houma A, Marro JP, Brun J. Lâcher de *Phanerotoma ocuralis* Kohl contre la pyrale des dattes, *Ectomyelois ceratoniae* Zeller, dans une parcelle expérimentale à Tozeur en Tunisie. Fruits. 1996;51:129-132.
4. Dhouibi MH. Les principaux ravageurs du Palmier Dattier et de la Datte en Tunisie. Brochure. Tunisie 1991, 64.
5. Mediouni J. Lutte génétique contre la pyrale des caroubes

Ectomyelois ceratoniae Zeller (Lepidoptères: Pyralidae) par le biais de la technologie des insectes stériles. Thèse de Doctorat en sciences agronomiques. Institut National Agronomique de Tunisie. 1881, 153.
6. Hached W, Ben Romdhane S, Sahraoui H, Grissa-Lebdi K. Control trials against *Ectomyelois ceratoniae* Zeller 1881 (Lepidoptera: Pyralidae) under controlled conditions and in citrus orchard. Journal of New Science 2018;49(3):2961-2970.
7. Zougari S, Attia S, Zouba A, Lebdi-Grissa K. Effectiveness of mass trapping and *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae) releases against *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae) in Tunisian oases. Biologia 2020. https://doi.org/10.2478/s11756-020-00628-2
8. Mamay M, Levent Ü, Ertan Y, Mahmut D, Ali İ. Efficacy of mating disruption technique against carob moth, *Apomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae) in pomegranate orchards in Southeast Turkey (Şanlıurfa). International Journal of Pest Management 2016;62:295-299.
9. Khoualdia O, Rhouma A, Marro JP. Utilisation de *Trichogramma cacoeciae* Marchal (Hymenoptera, Trichogrammatidae) contre la pyrale des dattes. Annales de l'INRAT. 1996;69:197-20.
10. Dang XL, Wen SY, He XF, Pang XF. M-PCR: a powerful method for rapid molecular identification of *Trichogramma* wasps (Hymenoptera: Trichogrammatidae). Insect Science. 2005;12:77-85.
11. Li LY. Worldwide use of *Trichogramma* for biological control on different crops: A survey" In *Biological control with Egg parasitoids*, Edited by: Wajnberg, E. and Hassan, SA. Wallingford: CAB International 1994, 37-53.
12. Moezipour M, Kafil M, Allahyari H. Functional response of *Trichogramma brassicae* at different temperatures and relative humidities. Bulletin of Insectology. 2008;61(2):245-250.
13. Herz A, Hassan SA, Hegazi E, Khafagi WE, Nasr FN, Youssef AI, *et al.* Egg parasitoids of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) in olive groves of the Mediterranean region. Biological Control. 2007;40:48-56.
14. Khoualdia, O, Rhouma A, Jarraya A, Marro JP, Brun J. Un trichogramme, nouveau parasite d'*E. ceratoniae* Zeller (Lep: Pyralidae) en Tunisie. Annales de l'Institut National de la Recherche Agronomique de Tunisie 1995;68:145-151.
15. Zouba A, Chermiti B, Chraiet R, Mahjoubi, K. Effect of Two Indigenous *Trichogramma* Species on the Infestation Level by Tomato Miner *Tuta absoluta* in Tomato Greenhouses in the South-West of Tunisia. Tunisian Journal of Plant Protection. 2013;8:87-106.
16. Hassan SA. Massenproduktion und Anwendung von *Trichogramma*. Produktion des Wirtes Sitotroga cerealella Oliv. Entomophaga. 1981;26:339-348.
17. Zouba A, Chermiti B, Kadri K, Fattouch S. Molecular characterization of *Trichogramma bourarachae* strains (Hymenoptera: Trichogrammatidae) from open field tomato crops in the South West of Tunisia. Biomirror 2013;4:5-11.
18. Lehmann P, Ammunet T, Barton M, Battista A, Eigenbrode SD, Jepsen J, *et al.* Complex responses of global insect pests to climate warming. Frontiers in the ecology and the environment. 2020;18(3):141-150.
19. Zouba A, Abbes K, Zougari S, Ben Hmida F, Chermiti B. Suitability of *Ectomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae) as host for two local *Trichogramma* species (Hymenoptera: Trichogrammatidae) and possible implications on biological control programs. Journal of oasis agriculture and sustainable development. 2021;3(1):45-53.
20. Ghosh E, Ballal CR, Verghese A. Temperature based differences in biological parameters of some potential species/strains of *Trichogramma*. Journal of biological Control. 20l7;31(2):82-89.
21. Reay-Jones FPF, Rochat J, Goebel R, Tabone E. Functional response of *Trichogramma chilonis* to *Galleria mellonella* and *Chilo sacchariphagus* eggs. Entomologia Experimentalis et Applicata. 2006;118:229-236.
22. Suh CPC, Orr DB, Van D. *Trichogramma* releases in North Carolina cotton: why releases fail to suppress Heliothine pests. Journal of Economic Entomology 2000;93(4):1137-1145.
23. Kabiri F, Frandon J, Voegele J, Hawlitzky N, Stengel M. Evolution of a strategy for inundative releases of *Trichogramma brassicae* Bezd. (Hym Trichogrammatidae) against the European corn borer *Ostrinia nubilalis* Hbn. (Lep. Pyralidae). In: Proceedings Second International Conference on Agricultural Pests. Association Nationale de Protection des Plants, Paris. 1990, 1225-1232.
24. Pizzol J, Pintureau B, Khoualdia O, Desneux N. Temperature-dependent differences in biological traits between two strains of *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae). Journal of Pest Science. 2010;83:447-452.
25. Lawson DS, Nyrop JP, Reissig WH. Assays with commercially produced *Trichogramma* (Hymenoptera: Trichogrammatidae) to determine suitability for ob-lique banded leafroller (Lepidoptera: Tortricidae) control. Environmental Entomology. 1997;26:684-693.
26. Lebdi-Grissa K, Ben Ayed N. Lutte biologique contre *Ectomyelois ceratoniae* sur grenadier par des lâchers de *Trichogramma cacoeciae*. AFPP 7ème Conférence Internationale sur les Ravageurs en Agriculture, Montpellier. 2005, 7.
27. Dhouibi MH, Hermi H, Soudani D, Thlibi H. Biocontrol of the carob moth *Ectomyelois ceratoniae* in pomegranate and citrus orchards in Tunisia. International Journal of Agriculture Innovations and Research. 2016;4(5):849-856.
28. Khoualdia O. Possibilités d'utilisation de la congélation rapide comme alternative au bromure de méthyle pour la désinfection des dattes. Mémoire de Mastère. Ecole Supérieure d'Horticulture et d'Elevage Chott-Mariam, Tunisia 2006, 61.
29. Hached W, Sahraoui H, Blel A, Lebdi Grissa K. Biological control of *Ectomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae) using the egg parasitoid *Trichogramma cacoeciae* Marchal (Hymenoptera: Trichogrammatidae) in a Tunisian citrus orchard. Journal of Entomology and Zoology Studies. 2021;9(3):98-104.
30. Ben Chaabane S, Mahjoubi K. Reduction in carob moth *Ectomyelois ceratoniae* (Lepidoptera, Pyralidae) populations by means of mass trapping pheromone and

Trichogramma cacoecia (Hymenoptera: Trichogrammatidae) releases in Tunisian oases. Journal of New Sciences. 2019;2(61):3866-3873.
31. Sayed SM, El Sayed G, Mahmoud SF, Elzahrany OM. Efficacy of *Bacillus thuingiensis* and indigenous *Trichogramma turkistanica* for controlling Lepidopterous pests of Taify pomegranate fruits. African Entomology 2015;23(2):443-450.
32. Seymour JE, Jones RE. Geographic variation in host instar and species preference of *Microplitis demolitor* (Wilkinson) (Hymenoptera: Braconidae) towards two of its native hosts, *Helicoverpa punctigera* (Wallengren) and *Helicoverpa armigera* (Hübner Lepidoptera: Noctuidae). Australian Journal of Entomology. 2001;40:245-248.
33. Bagheri A, Seyahooei MA, Fathipour Y, Famil M, Koohpayma F, Mohammadi-Rad A, Parichehreh S. Ecofriendly managing of *Helicoverpa armigera* in tomato field by releasing *Trichogramma evanescence* and *Habrobracon hebetor*. Journal of Crop Protection. 2019;8(1):11-19.
34. Usman M, Inayatullah M, Usman A, Sohail K, Shah SF. Effect of egg parasitoid, *Trichogramma chilonis*, in combination with *Chrysoperla carnea* and neem seed extract against tomato fruitworm, *Helicoverpa armigera*. Sarhad Journal of Agriculture. 2012;2(28):253-257.
35. Adly D, Nouh GM. Impact of combine releases of the egg parasitoid, *Trichogramma euproctidis* (Girault) and the entomopathogenic nematode, *Heterorhabditis bacteriophora* to control *Tuta absoluta* (Meyrick) in tomato greenhouses in Egypt. Egyptian Journal of Pest Control. 2019;29:91.
36. Basley K, Davenport B, Vogiatzis K, Goulson D. Effects of chronic exposure to thiamethoxam on larvae of the hoverfly Eristalis tenax (Diptera, Syrphidae). Peer J 6: e4258.
37. Graf N, Battes KP, Cimpean M, Dittrich P, Entling MH, Link M, Schäfer RB. Do agricultural pesticides in streams influence riparian spiders? Scie Total Environt 2019;660:126-135.
38. Yu DS, Byers JR. Inundative release of Trichogramma brassicae Bezdenko (Hymenoptera, Trichogrammatidae) for control of European corn borer in sweet corn. Canadian Entomologist. 1994;126:291-301.
39. Stevens PS. Host preferences of *Trichogrammatoidea bactrae fumata (Hym. Trichogrammatidae)* an egg parasitoid of leafrollers (Lep. Tortricidae). Entomophaga. 1995;40**:**379-385. https://doi.org/10.1007/BF02373726