



Effectiveness of mass trapping and *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae) releases against *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae) in Tunisian oases

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Abstract

This study was carried out to evaluate two biological methods for the control of *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae) which is considered to be the most important pest species in Tunisia. Mass trapping through 14 traps (water and delta) baited with sex pheromone, and *Trichogramma cacoeciae* releases with two doses: 25,000 *Trichogramma* (T)/ha (D1) and 50,000 T/ha (D2) were tested separately. Field trials were conducted during 2016 in oases cultivated by date palm trees and pomegranates. In this experiment the infestation rates for delta traps, water traps and control were 17.6, 32.9 and 87.3%, respectively. Therefore, mass trapping with 14 delta traps was the most effective on pomegranate fruits. After inundative releases of *T. cacoeciae* (D1: 25,000 and D2: 50,000 T/ha), the results revealed an impact of this parasitoid in inhibiting carob moth eggs hatching, and a significant reduction of the infestation rate of pomegranate fruits between 19.1 and 22.7% for both doses compared to control plot with 87.3%. However, these trials (mass trapping and *Trichogramma* releases) applied on date palm trees were not sufficient to limit economic losses.

Keywords *Ectomyelois ceratoniae* · *Trichogramma cacoeciae* · Mass trapping; water traps · Delta traps · Oases

Introduction

The date palm sector occupies an important place in the agricultural field in Tunisia. In fact, the palm groves located in the oases of southern Tunisia cover a total area of nearly 56 million ha with 5.2 million trees in 2016 which makes this sector the second after the olive oil sector (Observatoire National de l'Agriculture 2018). Indeed, Tunisia participates in the world date export market by 18.2% of the total value of exports with \$254 million (Observatoire National de l'Agriculture 2018). Besides, Tunisia has 31% of its date under organic production and there is a steadily increasing demand for organic foods (Hached 2019).

Pomegranate, *Punica granatum* L., has been cultivated since antiquity (Evreinoff 1949). This tree is well distributed in Tunisia from north to south as well in the coastal or mountainous areas, as the oases, because it is well adapted to the limestone soils, lack of water and arid climate (Mars and Marrakchi 1999). However, this large area of pomegranate trees provides low production (Dhoubi and Jammazi 1993, 1996). In ancient oases in the south of Tunisia, the traditional method is to plant the palms trees combined with fruit trees such as pomegranates.

Despite the abundance of pomegranate and palm productions, these two crops face many phytosanitary problems including lepidopterans. The carob moth *Ectomyelois ceratoniae* Zeller, 1839 (Lepidoptera: Pyralidae) is one of the most important pests in its larval stage (Mediouni and Dhouibi 2007; Krenn 2010) and is recognized as economically damaging several agricultural crops in many parts of the world such as Iran (Shakeri 2004; Norouzi et al. 2008; Sobhani et al. 2015; Ahmadi et al. 2016), Kingdom of Saudi Arabia (Al Barty 2014), Turkey (Ozturk et al. 2011; Mamay et al. 2016), United States of America (Nay 2005; Vetter et al. 2006), and the Mediterranean region such as Spain (García-Martínez et al. 2017), Italy (De Stefani 1920), Morocco (Madkouri 1978; Bouka et al. 2001) and Algeria (Ouamane et al. 2017; Roumani et al. 2018). The carob moth has been reported

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on 43 hosts from 18 plant families. Twenty-one of these plants are economically important as agricultural commodities, 15 are ornamental plants and seven can be considered having multiple uses. The vast majority of hosts are tree species, and the families with the most species attacked are the Fabaceae and the Rosaceae (Perring et al. 2016). The majority of damage caused by *E. ceratoniae* is due to fruit infestations (Norouzi et al. 2008; Sobhani et al. 2015; Ahmadi et al. 2016). This pest is commonly found in carobs *Ceratoniasiliqua* (L.) (Gothilf 1984), pistachios *Pistacia vera* (L.) (Mehrejjad 1993), almonds *Prunus dulcis* (Mill) (Gothilf 1984), figs *Ficus carica* (L.) (Shakeri 1993), walnuts *Juglans nigra* (L.) (Balachowsky 1972), citrus *Citrus sinensis* (L.) (Gothilf 1984), dates *Phoenix dactylifera* (L.) (Echlin 1982) and pomegranates *Punica granatum* (L.) (Al-Izzi et al. 1985; Mamay et al. 2016). In Tunisia, this ubiquitous species is the most important insect on pomegranate fruits (Braham 2015), citrus (Hached et al. 2018) and date palms (Khoualdia et al. 1996).

In order to obtain stable or higher yields, the management of this pest is crucial. However, the control of *E. ceratoniae* in Tunisia relies heavily on applications of chemical pesticides such as Abamectine and Emamectine-benzoate, which is commonly used to control carob moth in citrus orchards (Hached et al. 2018). Although, the use of pesticides would yield benefits such as the highest efficacy in reducing the infestation rate of fruits (Fornasiero et al. 2017) and the specificity of active molecules (Napoleao et al. 2018), the intensive use of pesticides has led to resistance in many insect species (Bass et al. 2015; Dermauw et al. 2018), threat to non-target organisms such as predatory wasps (Barros et al. 2014), lacewings (Amarasekare et al. 2015), hoverflies (Basley et al. 2018), ground beetles (Sharavari et al. 2015) and spiders (Graf et al. 2019), and toxicity to human health and environment (Nicolopoulou et al. 2016; Kim et al. 2017).

Besides, many chemical products have been shown to act on the nervous and reproductive systems of humans (Grewal et al. 2017), causing problems into the future (Jayaraj et al. 2016; Mahmood et al. 2016). As we know, in many countries there are no plans to ban pesticides from fields and other areas. Therefore, technical, economic and environmental factors are forcing to adopt new sustainable methods and there has been an increasing interest in controlling carob moth by alternative control tactics such as biological control agents (Frank 2010). Mass trapping and *Trichogramma* releases seem to be good alternatives to control the carob moth without damaging the ecosystem (Mamay et al. 2016; Mehrijardy et al. 2016; Gagnon et al. 2017; Ouamane et al. 2017). In fact, mass trapping combined with sex pheromone of pest is being developed in greenhouses against the tomato pinworm *Tuta absoluta* Meyrick, 1917 (Abbes et al. 2012; Cherif et al. 2017), in citrus orchards against the mediterranean fruit fly *Ceratitis capitata* Wiedemann, 1824 (Harbi et al. 2018; Hafsi et al. 2015) and in pomegranate orchards against the carob moth *E. ceratoniae* (Dhouibi et al. 2016).

The parasitoid *Trichogramma cacoeciae* Marchal, 1927 is known as biocontrol agents of many pests including lepidopteran species in the world (Desneux 2010; Chailleux et al. 2013). In Tunisia, it is used frequently against *T. absoluta* and *E. ceratoniae*. The releases of about 20 to 30 females of *Trichogramma* per tomato plant in greenhouses and 25,000 *Trichogramma* (T)/ha proved to be effective in controlling tomato fields against *T. absoluta* (Cherif et al. 2018; Zouba et al. 2013) and 25,000 T/ha (Dhouibi et al. 2016) and 16,000 T/ha (Hached 2019) to control *E. ceratoniae* in citrus orchards.

The present work was undertaken to evaluate the efficiency of mass trapping of *E. ceratoniae* using two types of traps water and delta. Furthermore, we performed inundative releases of *T. cacoeciae* to reduce damage of the carob moth in organic date palm orchards in the south-west of Tunisia.

Materials and methods

Experimental plots

Experiments were conducted during 2016 in three organic orchards including pomegranates and date palms in Nefleyette region belonging to governorate of Tozeur located in the south-west of Tunisia. Each orchard had an area of 2 ha, with date palms spaced at 8 m within and between rows. A total of 120 palms of Deglet Nour variety and 35 of Tounsi cultivar of pomegranate trees/ha per orchard were planted since 1975. Two orchards were divided each into four plots with an area of 0.5 ha. Plots were separated from each other by palms as windbreaks to make sure that treatments did not reach the other plots. Control treatment was conducted in the third orchard with an area of 1 ha including pomegranate and date palm trees. Experimental orchards received no insecticide treatment during the release period.

Climatic conditions

Climate data were recorded from weather station located in Tozeur. This region has a desert climate with low precipitation; a maximum rainfall registered from 2 May 2016 until 30 October 2016 was 4.6 mm (Fig. 1). For the same period, the mean temperature as well as the relative humidity varied from 19.7 to 34.9 °C and 31.5 to 68.2%, respectively (Fig. 1).

Mass trapping

Mass trapping trails were tested in an area of 2 ha, both delta and water traps were used in this experiment with a density of 14 traps per ha. Therefore, the tested area was divided into four equivalent replications: (1) and (2) for water traps on pomegranates and date palm trees, (3) and (4) for delta traps

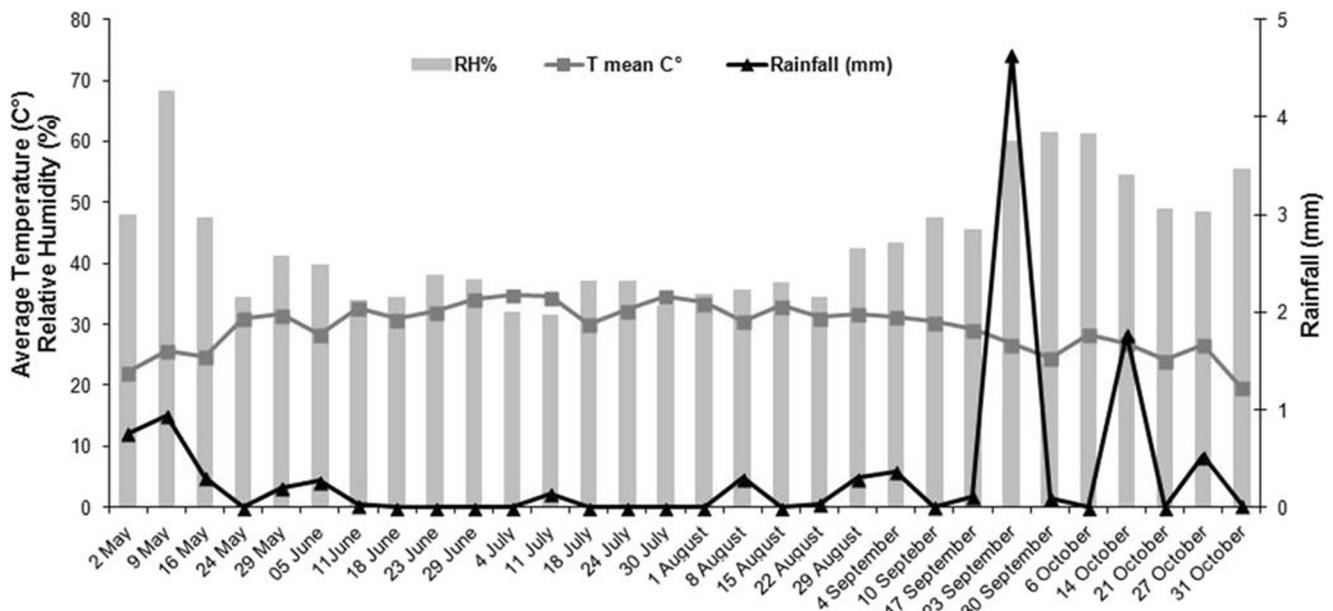


Fig. 1 Climograph of weekly mean temperature (C°), rainfall (mm) and relative humidity (%) during the monitoring period for *E. ceratoniae* in governorate of Tozeur (Tunisia) from 02 May until 31 October 2016

on pomegranate and date palm trees. Delta traps (ISCA) were composed of sticky cardboards 19.5 cm long, with a triangular cross-section of 10 × 10 × 10 cm and a 3 × 3 × 3 cm triangular entrance at each end; two of the three internal sides were coated with a sticky surface to retain trapped moths. Traps were stapled to a small tree trunk at a height of 1.5 m oriented in the same cardinal direction and checked once a week, the pheromone baits and sticky cardboards were changed with fresh ones every month.

A water trap used in this experiment was composed of a plastic recipient with a capacity of 2 L (21 × 15 × 8.5 cm) containing 1.5 L of water mixed with 30 ml of detergent and combined with sex pheromone of *E. ceratoniae* that was stapled to the trunk of the tree of each host plant.

Trichogramma releases

Releases tests were conducted in a 2 ha area. The parasitoid *T. cacoeciae* tested in the experiment has been already commercialized in Tunisia to control lepidopteran pests. Cardboards (5 × 1 cm²) containing eggs of *Ephestia kuehniella* Zeller, 1879 parasitized by this parasitoid were provided by a private company (ControlMed, Tunisia). Four consecutive releases of *T. cacoeciae* spaced four weeks each were done. Two doses, 25,000 (D1) and 50,000 (D2) T/ha, were tested. The trial was conducted in four equivalent blocks containing each 60 date palm trees and 17 pomegranate trees (an area of 0.5 ha): (1) 25,000 T/ha for pomegranate trees, (2) corresponding to 25,000 on date palm trees, (3) 50,000 T/ha for pomegranate trees and (4) releases of 50,000 T/ha were done on date palm trees. A total of 50,000 and 100,000 T were

released, respectively for D1 and D2 on 04/07, 05/08, 25/08 and 02/09 in 2016. For the four experimental plots one delta trap combined with sex pheromone of females of the carob moth was placed for monitoring of *E. ceratoniae* male flight.

In order to evaluate the efficacy of releases of *Trichogramma* in parasitizing *E. ceratoniae* eggs, a weekly sampling of 10 pomegranate fruits was done randomly from 4 July 2016 until 16 September 2016. Two hundred date fruits were collected randomly from five date palm trees from 2 September 2016 until 4 October in 2016. Then, samples were examined in the laboratory under binocular microscope (Leica® model MS5) to record the number of parasitized eggs of *E. ceratoniae*. To control the emergence rate of *Trichogramma*, after each release, 10 cardboards of 1000 T were taken randomly from each experimental plot and the numbers of hatched and unhatched eggs were counted. The emergence and parasitism rate were calculated according to the following formulas:

$$\text{Emergence rate (\%)} = \frac{\text{number of hatched eggs of } E. \text{ kuehniella}}{\text{total number of released eggs}} * 100$$

$$\text{Parasitism rate (\%)} = \frac{\text{number of parasitized eggs of } E. \text{ ceratoniae}}{\text{total number of eggs recorded}} * 100$$

Infestation by *E. ceratoniae*

Infestation rate was already determined on pomegranate and date palm fruits. The effectiveness of mass trapping through water and delta traps (with 14 traps/ha) and inundative releases of *T. cacoeciae* with the two doses 25,000 and 50,000 T/ha was determined by a weekly counting of the total

number of pomegranate fruits from five trees selected at the beginning of the experiments. Therefore, the total number as well as the number of infested fruits by *E. ceratoniae* was registered from 13 May 2016 until 16 September 2016. In addition, two-hundred date fruits/week/plot were collected randomly from five date palm trees from 2 September 2016 until 4 October 2016. All samples were examined under a binocular microscope in the laboratory. Every observed stage of *E. ceratoniae* referred to an infested fruit.

The reduction rate was calculated according to Abbott's formula as follows:

$$\text{Reduction rate} = [(A-B/A)*100]$$

A is the mean infestation of pomegranate and date fruits per week in control treatment and **B** is the mean infestation of pomegranate fruits per week in treated plot (Abbott 1925).

The infestation rate for both host plants was calculated according to the following formula:

$$\text{Infestation rate} = (N_A/N_B)*100$$

For pomegranate:

N_A is the number of infested pomegranate fruits per tree and N_B is the total number of pomegranate fruits per tree.

For date:

N_A is the number of infested fruits and N_B is the total number of fruits.

Statistical analyses

Graph pad Prism version was used to perform all statistical analysis. The obtained data corresponding to the parasitism rate of *E. ceratoniae* as well as the effectiveness of mass trapping and *T. cacoeciae* releases were subjected to repeated measures analysis of variance (GLM procedure). Additional one-way ANOVA followed by Tukey post hoc tests at $p = 0.05$ inside each treatment was carried out.

Results

Mass trapping in pomegranate and date palm orchards

Male catches on pomegranate trees

Catches data of *E. ceratoniae* males for both tested traps, water and delta traps, are presented in Fig. 2 and showed that during July and August the number of male catches was

higher for delta traps compared with water traps. During the catching period, males started to appear with low density for the first two weeks with the two tested traps. Water and delta traps caught almost the same number of *E. ceratoniae* males. During the next period, the inspection of traps showed that water traps kept the same potential of catching moths, however, the catches of males through delta traps were higher compared with water traps. A total of 152 and 483 *E. ceratoniae* males/trap were caught by water and delta traps, respectively, with 14 traps/ha. The mean catches of the carob moth males reached 9.7 and 31.4 males/trap for water and delta traps, respectively (Fig. 3). Therefore, the assessment of trap catches showed a significant difference in capturing males between the two tested traps ($p = 0.0002$).

Male catches on date palm trees

In autumn-winter during maturity period, pheromone traps captured a total of 79 and 134 males/trap for water and delta traps, respectively. A maximum of captured males was recorded on 18 October with 16 and 27 males/trap for water and delta traps, respectively (Fig. 4). During the study period, the mean catches of males were significantly different between water and delta traps ($p = 0.0070$); they reached 11.3 and 19.1 males/trap for water and delta traps respectively (Fig. 5).

Mass trapping efficiency in reducing *E. ceratoniae* infestation on pomegranate and date palm fruits

Pomegranate fruits

Regular sampling of pomegranate fruits in the tested plots indicated that first infested fruit were recorded on 27 May 2016 and the percentage of infestation was about 3.8% in the control plot on the same date. However, our study showed that the percentages of attacked fruits were 0% for both trap types: water and sticky delta trap on the same date of sampling.

Infestation increased on 2 June 2016 to 2.5 and 2.7% for water and delta traps, respectively. Results showed that damages recorded on pomegranate fruits caused by *E. ceratoniae* were significantly different between the studied plots (water and delta trap plots) compared with the control ($F_{(2, 28)} = 17.77$; $p < 0.0001$) (Fig. 6). During harvest, control treatment showed maximum fruit infestation 87.3% compared with 32.9 and 17.6% for water and delta traps, respectively. Therefore, water and delta traps reduced the infestation caused by *E. ceratoniae* to 62.3 and 79.8%, respectively, compared with the untreated plot.

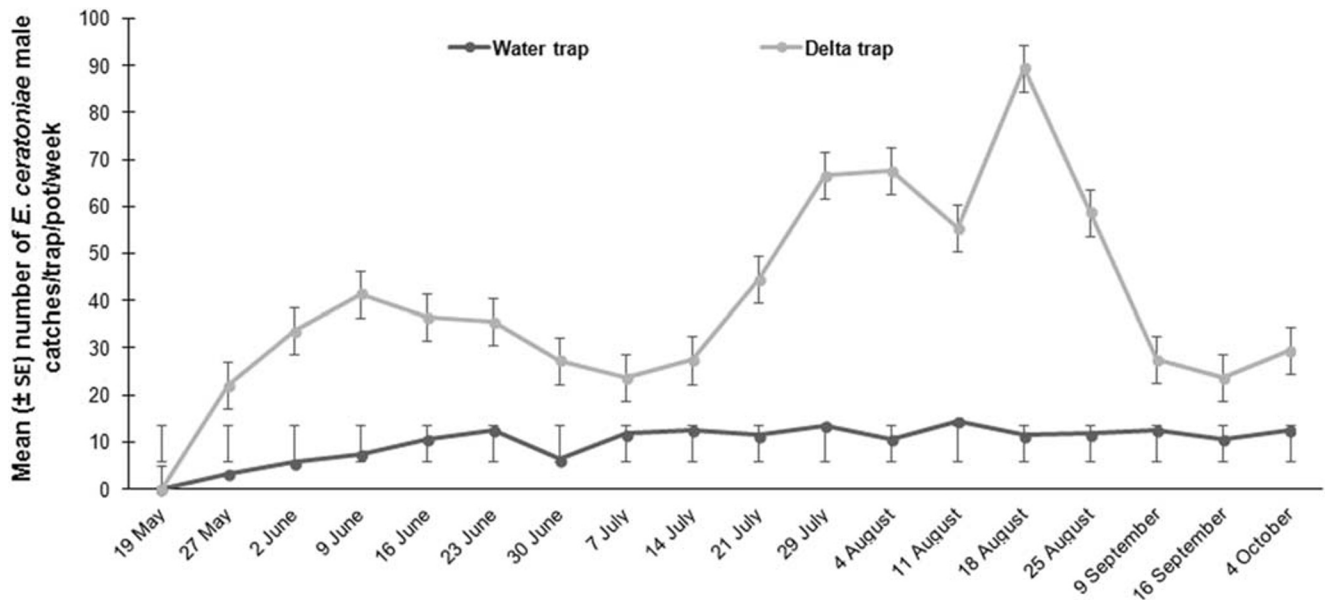


Fig. 2 Weekly catches of *E. ceratoniae* male per Water and Delta traps on pomegranate trees from 19 May until 04 October in Nefleyette oases during 2016

Date palm fruits

Contrary to pomegranate fruit results, the current experiment of mass trapping efficiency in reducing infestation caused by the carob moth on date palm fruit seem to be not satisfying. The regular sampling of 200 date palm fruits per week/plot from 2 September to 29 October 2016 (Fig. 7) showed that there was no significant difference between plots ($F_{(2, 4)} = 1.044$; $p = 0.4317$), the highest infestation of date fruits reached 24% in delta plot compared to 18.9 and 18.5% for water trap and control plot, respectively.

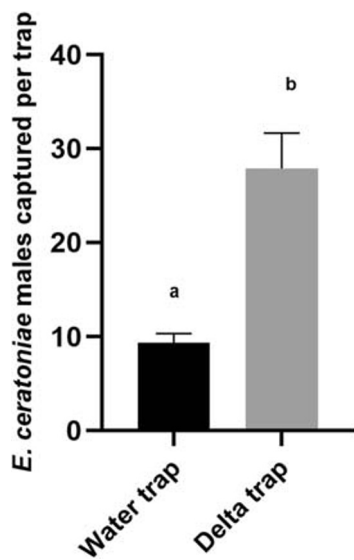


Fig. 3 Number of *E. ceratoniae* males captured per different trap types (Delta and Water traps) on pomegranate trees in Nefleyette oases during 2016. Different letters above the bars indicate significant differences ($p \leq 0.05$; Tukey’s test)

Trichogramma releases

Emergence rate

Emergence rates of female *T. cacoeciae* from *E. kuehniella* eggs varied from 72.7 to 94.8% for both tested doses, 25,000 and 50,000 of T/ha, respectively. For both pomegranate and date palm trees emergence of *T. cacoeciae* was successful.

Male captures

Pomegranate

Results of male catches of *E. ceratoniae* through pheromone traps from 4 July 2016 until 9 September 2016 on pomegranate trees (Fig. 8) showed the presence of the pest during all the study period. For the first release we recorded 3, 5 and 6 males/trap for 25,000 (D1), 50,000 T/ha (D2) and control plots, respectively. A major peak of adult males of *E. ceratoniae* was registered during the third release of *Trichogramma* (29 August 2016) with 4, 3 and 16 males/trap for D1, D2 and control, respectively. Tukey’s test showed a significant difference between the treated plots and control ($F_{(1,265, 10,12)} = 19.78$, $p = 0.0008$) and ANOVA test showed a similarity in capturing carob moth males after *Trichogramma* releases for both tested doses ($p = 0.8958$).

Date palm

From 2 September until 4 October 2016 we recorded lesser moth catches than captures on pomegranate trees (Fig. 9). Fluctuation of *E. ceratoniae* males showed maximum catches on 23 September 2016 with 4, 5 and 7 males/trap for D1, D2

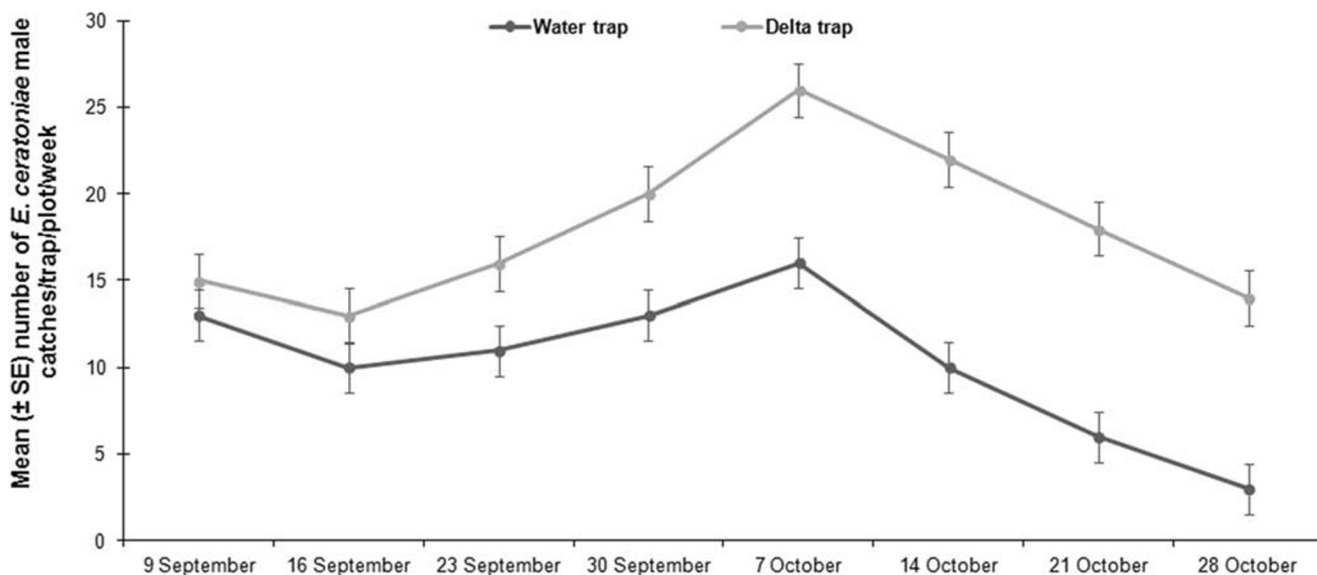


Fig. 4 Weekly catches of *E. ceratoniae* male per Water and Delta traps on date palm trees from 9 September until 29 October in Nefleyette oases during 2016

and control plots, respectively. ANOVA showed a significant difference between the different tested plots ($F_{(1,727, 6,907)} = 21, p = 0.0014$).

Parasitism rate

Pomegranate fruits

The presence of *E. ceratoniae* male moths in the target area was detected three days after fruit emergence, initiating the

first release of *T. cacoeciae* in the experimental plots (D1 and D2).

A sample of 10 pomegranate fruits per tested plot was taken before *T. cacoeciae* release on 4 July 2016 and examined under binocular microscope. Results showed that this parasitoid was present in Nefleyette orchard with low density. Parasitism rate was about 3.9 and 6.1% of parasitized *E. ceratoniae* eggs (Fig. 10). Parasitism rate increased over the consecutive releases (after the second and third releases) for both tested doses. A maximum parasitism rate was recorded during the last release (3rd) in pomegranate fruits with 41.4% and 80.2% for 25,000 and 50,000 T/ha,

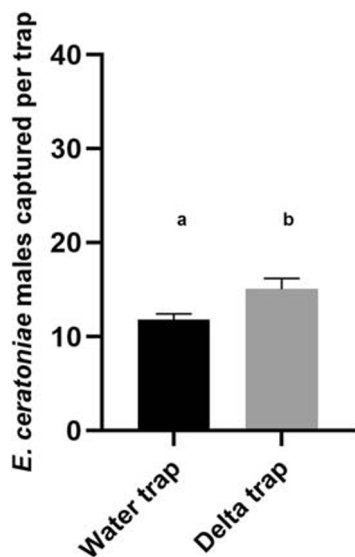


Fig. 5 Number of *E. ceratoniae* males captured per trap types (Delta and Water traps) on date palm trees in Nefleyette oases during 2016. Different letters above the bars indicate significant differences ($p \leq 0.05$; Tukey's test)

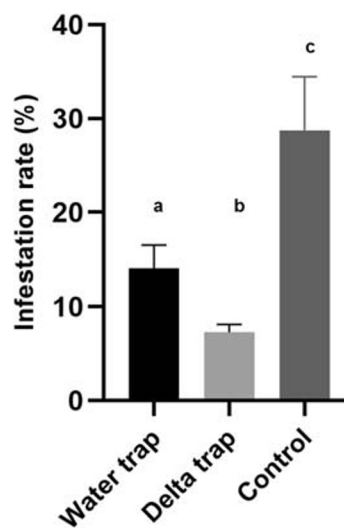


Fig. 6 Infestation rate of pomegranate fruits in Nefleyette oases during 2016. Different letters above the bars indicate significant differences ($p \leq 0.05$; Tukey's test)

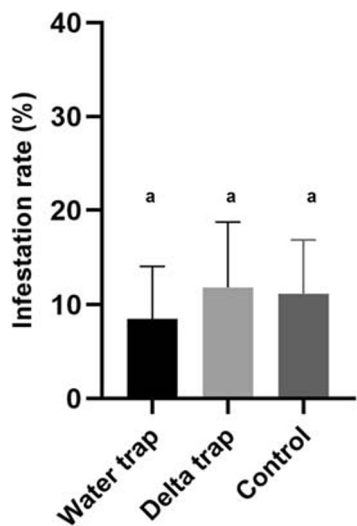


Fig. 7 Infestation rate of date palm fruits in Nefleyette oases during 2016. Different letters above the bars indicate significant differences ($p \leq 0.05$; Tukey’s test)

respectively. Data analyses showed a p value of 0.0039 proving a significant difference between 25,000 and 50,000 T/ha releases in parasitizing carob moth eggs.

Date palm fruits

Samples of date fruits taken on 2 September 2016 showed 0% of parasitism of carob moth’s eggs for each experimental plot. One week after *Trichogramma* release, maximum rate of parasitized eggs of carob moth was 6.7% for D1 compared to 18.3% for D2 (Fig. 11). There was no difference observed

between 25,000 and 50,000 T/ha in parasitizing eggs of *E. ceratoniae*.

Infestation rate

After three releases of the parasitoid on pomegranate trees, a significant reduction in carob moth infestation on pomegranate fruits was observed between the treated and control plots ($F_{(1,006, 9,052)} = 25.93, p = 0.0006$). Nevertheless, no statistical difference was recorded between released doses ($p = 0.1557$) (Fig. 12a). Indeed, releases of D1 and D2 on date palm trees were not sufficient to reduce damages of date fruits ($F_{(1,06, 3,179)} = 0.3136, p = 0.6250$) (Fig. 12b). Pomegranate infestation increased over time, the highest infestation rate of fruits was recorded during harvest in control plot with 87.3% compared to 22.7 and 19.1% after *Trichogramma* release with the two tested doses D1 and D2, respectively. Therefore we registered a reduction rate of 74 and 78.1% for D1 and D2, respectively. The infestation rate is about 17.5 and 19% after release of 25,000 and 50,000 T/ha, respectively (Fig. 12b).

Discussion

Mass trapping

Male catches

This work showed a considerable capacity of capturing *E. ceratoniae* males through water and sticky delta traps in

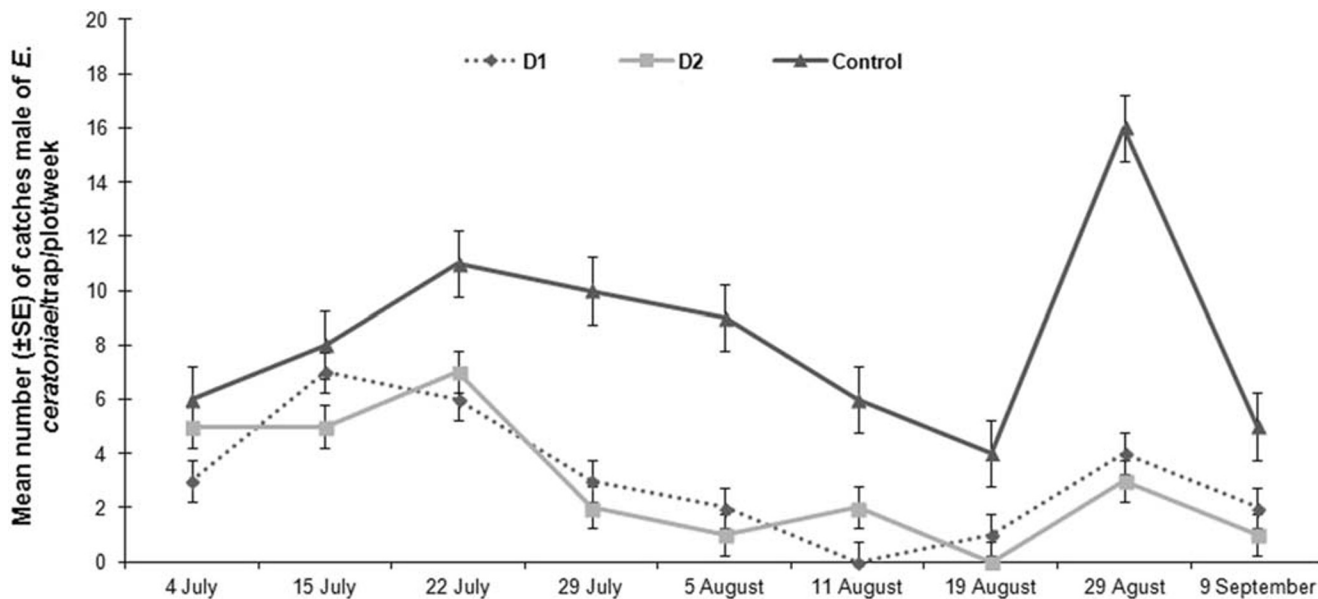


Fig. 8 Weekly captured males of *E. ceratoniae* in pomegranate plots after 25,000 T (D1) and 50,000 T (D2) releases during the period of study of 2016

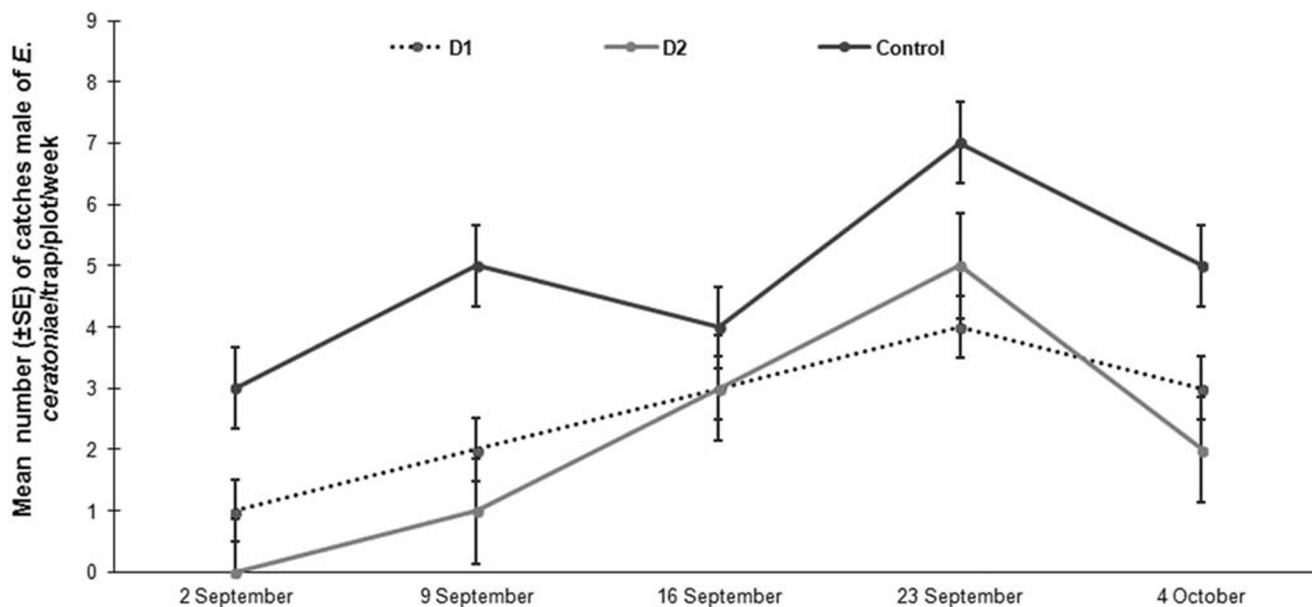


Fig. 9 Weekly captured males of *E. ceratoniae* in date palm plots after 25,000 T (D1) and 50,000 (D2) releases during 2016

Tozeur orchards from May until October 2016. Results showed that mass trapping requires the use of a highly efficient pheromone trap in order to remove most of the males before they encounter females and mate (El Sayed et al. 2006). Delta traps had captured significantly more males compared to water traps. A similar study was conducted in Iran, where Mehrjardy et al. (2016) showed that captured moths by the same type of traps, were 841 and 722 individuals in pomegranate and fig orchards, respectively. Several studies showed that delta traps were commonly used for controlling lepidopteran pests such as the codling moth, *Cydia pomonella* Linnaeus, 1758 (Willson and Trammel 1980; El Sayed et al. 2006), tomato leafminer *T. absoluta* (Aksoy and Kovanci 2016), lesser date moth

Batrachedra amydraula Meyrick, 1916 (Levi-Zada et al. 2017) *Lobesia botrana* Denis & Schiffmüller, 1775 (Rayegan et al. 2016) and gypsy moth *Lymantria dispar* Linnaeus, 1758 (Charelton and Cardé 1990).

To our knowledge, no published literature was found on testing water traps to control *E. ceratoniae* on pomegranate and date palm orchards. The potential of male captures of *E. ceratoniae* by water traps can be reduced by the evaporation problem under hot weather conditions in the summer season, when temperature exceed 33 °C during July and August in the south west of Tunisia (Fig. 1). Chermiti and Abbes (2012) reported the effective use of large traps containing 5 L of water for mass trapping high populations of *T. absoluta* at a density of

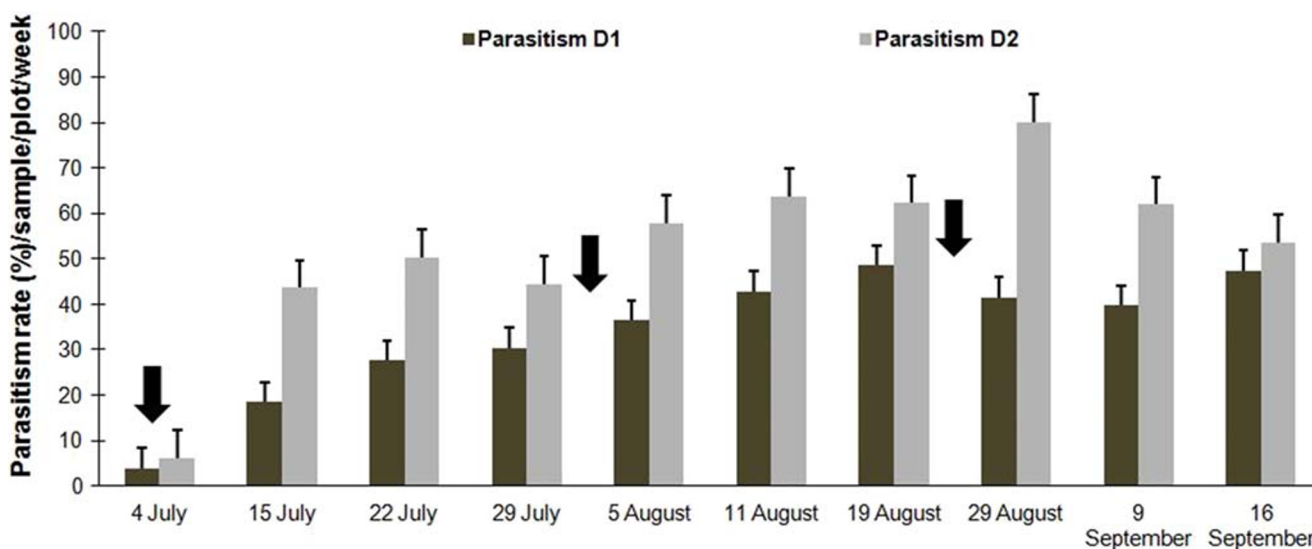


Fig. 10 Weekly parasitism rate of *E. ceratoniae* eggs after 25,000 (D1) and 50,000 (D2) T/ha releases on pomegranate trees in Nefleyette orchards during 2016. Arrows indicate release's date of *Trichogramma* for both tested doses

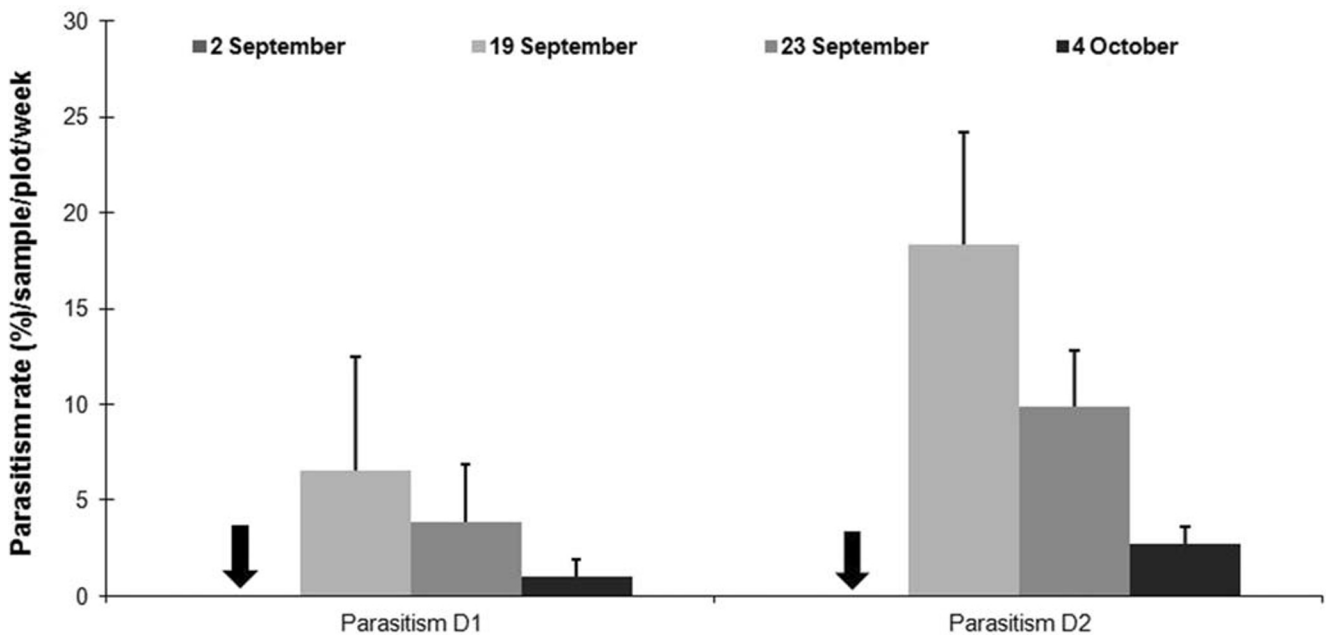


Fig. 11 Weekly parasitism rate of *E. ceratoniae* eggs after releases of 25,000 (D1) and 50,000 (D2) T/ha on date palm trees in Nefleyette orchards during 2016. Arrows indicate releases date of *Trichogramma* for both tested doses

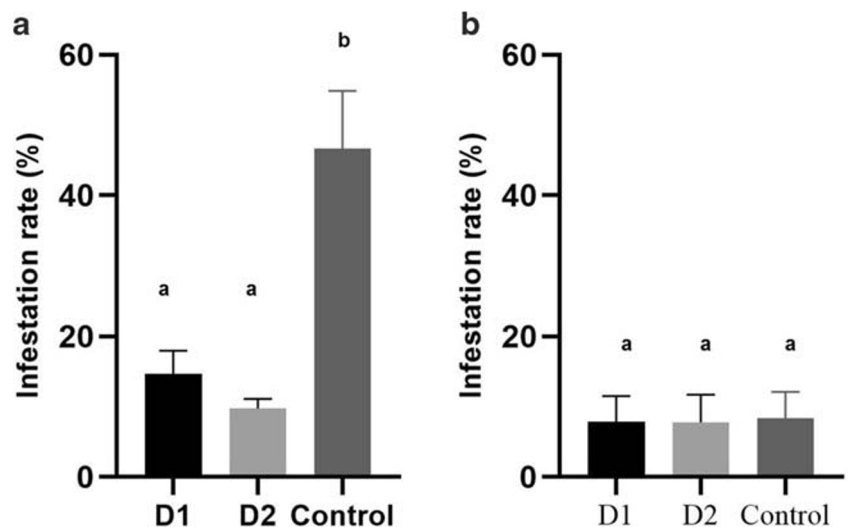
32 traps per ha in open- tomato fields in Tunisia. These authors recommended the use of mass trapping through water traps in case of important population of the tomato pinworm that allows capturing of a large number of males of *T. absoluta* without causing saturation problems in the recipient (USDA APHIS 2011).

Infestation

Water and sticky delta traps, were used during all the study period; the latter one seemed to be sufficient in reducing *E. ceratoniae* population and limiting damage of fruits in orchards. In fact, our results showed high reduction of

infested pomegranate fruits with 62.3 and 79.8% for water and delta traps, respectively, during harvest. However, no efficacy of any trap type was statistically proved on date palm fruits. Stand-alone mass trapping has been tested for control of a wide range of insect pests such as *C. capitata* (Diptera: Tephritidae) (Andrés and Ferran 2013), *Rhynchophorus ferrugineus* Olivier, 1791 (Coleoptera, Dryophthoridae) (Hamidi et al. 2013), and *T. absoluta* (Lobos et al. 2013). In some programs, mass trapping showed potential for pest management with a significant reduction in target pest population density. Zhang et al. (2002) showed that mass trapping baited with sex pheromone had reduced damages on *Sophora japonica* (L.)

Fig. 12 Infestation of pomegranate (a) and date fruits (b) after releases of 25,000 (D1) and 50,000 (D2) T/ha compared with control plot in Nefleyette orchards during 2016. Different letters above the bars indicate significant differences ($p \leq 0.05$; Tukey’s test)



caused by Chinese tortrix *Cydia trasi* Meyrick, 1928 by 87–96%. Another study conducted with the mass trapping method was conducted on pistachio orchards in Turkey showed that the infestation of *Kermania pistaciella* Amsel, 1964 (Lepidoptera Oinophilidae) in twigs decreased from 50.2 to 5% (Yanik and Yildirim 2016).

In a similar study, Ben Chaabane and Mahjoubi (2019) confirmed that the density of 6 and 15 traps/ha on pomegranate and date palm trees can decrease infestation rate to 4% and 3.2% compared to 20 and 15%, respectively, in control plot during 2018. Another study conducted on pomegranate orchards in northern of Tunisia proved that a density of 10 delta traps per hectare can reduce significantly infestation rate of pomegranate fruits (Dhouibi et al. 2016). Unfortunately, there is no information on how to improve the effectiveness of water traps in catching *E. ceratoniae* in date palm and pomegranate orchards in Tunisia. However, efficiency of mass trapping through water traps was done in monitoring of *T. absoluta* in greenhouses and open field tomato crops (Cherif et al. 2017; Chermiti and Abbes 2012; and Abbes et al. 2012). Thus, our work is the first documented experiment in using water traps as a new alternative to control *E. ceratoniae*. Management of the carob moth population on pomegranate fruit by mass trapping seemed to be satisfying, but no efficiency was recorded on date palm fruits that could be explained by climatic conditions as explained before. In USA, date fruits infested with larvae can cause damage reaching 10–40% to the harvestable date palm orchards each year (Warner et al. 1990; Nay 2005). Bouka et al. (2001) reported 30% yield losses in Morocco for the same host plant. In Tunisia, previous studies showed that this pest causes economic losses in pomegranate fruits exceeding 40% (Braham 2015). It is also a major field pest of pomegranate in Iraq, where the mean percentage of infested fruits increased from 20% to more than 80% (Al-Izzi et al. 1985). Our results demonstrated that mass trapping was not suitable for reducing the density of infested date fruits. However, it was successfully applied in other countries including, among others, Turkey (Demirel 2016) and Iran (Mehrdary et al. 2016) as the effectiveness on pomegranate fruits was more apparent. Knipling (1979) recommended that for successful mass trapping, more than 95% male annihilation may be required to control high populations of lepidopteran pests.

Trichogramma release

Few studies were conducted to test the impact of parasitoid releases on date palm in Tunisia. However, some studies combined *Trichogramma* releases with other trials. Ben Chaabane and Mahjoubi (2019) tested the efficacy of *Trichogramma* releases combined with mass trapping on date palm and pomegranate trees. Usman et al. (2012) investigated the efficiency of *Trichogramma chilonis* Ishii, 1941 combined with

Chrysoperla carnea Stephens, 1836 and neem extract against tomato fruit worm *Helicoverpa armigera* Hübner, 1808. Hwang et al. (2010) studied the effect of a combined treatment of *Bacillus thuringiensis* Berliner, 1915 and *Trichogramma ostrinae* Pang & Chen, 1974 to control *Plutella xylostella* Linnaeus, 1758. Also, *Trichogramma pretiosum* Riley, 1879 releases can be combined with pesticides to protect cotton (*Gossypium hirsutum*) (Bastos et al. 2006).

Male captures

Male catches of *E. ceratoniae* were high in all control and treatment plots in 2016. Most of the moths were trapped on 29 August with 16 males/trap in control plot. For both host plants, a significant reduction of male captures was observed between tested doses and control. However, no difference between D1 and D2 in catching carob moth males by delta traps. In a similar study Ben Chaabane and Mahjoubi 2019 mentioned 7 males captures of *E. ceratoniae* per trap after mass trapping and *T. cacoeciae* release in the south-ouest of Tunisia during 2018. Another study was conducted for testing the efficiency of *T. pretiosum* on *Spodoptera frugiperda* J.E. Smith, 1797 male captures which decreased during the trapping period with a total of 60 moths captured in the study period (Figueiredo et al. 2015).

Parasitism rate

Samples taken on 4 July showed very low number of parasitized eggs of carob moth. This is due to the natural parasitism, which proves the existence of parasitoids in date palm orchards of Tozeur with very low densities. Results showed that parasitism rate increased over releases up to 41.4 and 80.2% of parasitized *E. ceratoniae* eggs on pomegranate fruits for D1 and D2, respectively. Mertz et al. (1995) showed that the parasitism rate of *Trichogramma brassicae* Bezdenko, 1968 was between 30% and 50% when tested on *Ostrinia nubilalis* Hübner, 1796 eggs.

Parasitism rates reported for previously tested parasitoid species may be influenced by the number of released parasitoids and spacing among release points (Agamy 1994; Sarhan et al. 2015) given that species of *Trichogramma* are characterised by their reduced capacity of flight, and by the fact that they reach their host eggs by walking and jumping (Chailleux et al. 2013).

Infestation rate

The dose D1 (25,000 T/ha) seems to be suitable to reduce the impact of the carob moth on pomegranate fruits since there was no significant difference recorded for the two tested doses on pomegranate losses. Interestingly, *Trichogramma* egg parasitoids released

would obviously enhance the control performance against many lepidopteran species in Tunisia such as *T. absoluta* and *E. ceratoniae* (Ksentini et al. 2010; Lebdi-Grissa et al. 2010; Abbes et al. 2014). This was successfully applied in other countries including, among others, Egypt (Rizk 2016), USA (Salas et al. 2019), Pakistan (Hussein et al. 2015), and Canada (Dionne et al. 2018). Thus, *Trichogramma* are generally polyphagous parasitoids (Smith 1996). Several studies demonstrated their preference for some host species (Hassan 1989; Attic et al. 2001, Goulart et al. 2011). However, the ability of *Trichogramma* to select and parasitize eggs from the target host is necessary for the success of biological control programs (Pak 1988; Steidle et al. 2001). Zouba (2016) mentioned that females of *T. cacoeciae* and *Trichogramma bourarachae* Pintureau & Babault, 1988 showed a preference for parasitism over *E. ceratoniae* eggs compared to *T. absoluta* eggs with 90% of emergence for both pests. The use of *T. cacoeciae* females has just been targeted for protecting tomato groves (Zouba et al. 2013; Cherif and Grissa-Lebdi 2013; Cherif et al. 2018; Abbes et al. 2014), citrus (Dhouibi et al. 2016) and pomegranate orchards (Lebdi-Grissa and Ben Ayed 2005). About 75% reduction of infestation was achieved by releasing 40 adults of *T. cacoeciae* per plant in greenhouse tomato crops (Zouba and Mahjoubi 2010). In the south of Tunisia, releasing 25,000 of either *T. cacoeciae* or *T. bourarachae*, respectively, induced a reduction of 78% and 87% of damages in protected tomato greenhouses (Zouba et al. 2013). Therefore, the use of *T. cacoeciae* in pomegranate decreased fruit infestation from 13.2% in untreated plot compared with 2.4% in a treated plot (Lebdi-Grissa and Ben Ayed 2005). Indeed, Khoualdia et al. (1996) showed, through palm grove trials, that the parasitization rate on *E. ceratoniae* eggs averaged 78.7% in the release plots by *T. cacoeciae* and only 45% in date palm orchards of Algeria with *Trichogramma embryophagum* Hartig, 1838 against *E. ceratoniae* (Doumandji-Mitiche 1985; Doumandji-Mitiche and Idder 1986).

Conclusion

The present work showed the effectiveness of some biological methods that can be a promising strategy to control *E. ceratoniae* population in Tunisian oases. A biological control through mass trapping using water and delta traps combined with sex pheromone showed high efficacy in catching *E. ceratoniae* males as well as reducing pomegranate fruits damages during all the study period. However, statistical analyses did not show a significant efficiency on date palm fruit

damages comparing with control treatments due to the early harvest during 2016. Therefore, females of *T. cacoeciae* with 25,000 T/ha seem to be a promising alternative limiting the carob moth population.

Overall, *Trichogramma* egg parasitoids released in combination with mass trapping and pheromone-mediated management tactics would obviously enhance the control performance against *E. ceratoniae* in Tunisia. Therefore, it would be interesting to survey neighboring countries, Algeria, Morocco, Libya, for the existence of *E. ceratoniae*. Many experiments should be applied in future to reach the adequate trials including density of mass trapping and *Trichogramma* release for an Integrated Pest Management (IPM) against this pest in Tunisian oases.

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Compliance with ethical standards

Disclosure statement The authors report no conflict of interest.

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