

Field releases of *Trichogramma cacoeciae* (Marchal, 1927) (Hymenoptera: Trichogrammatidae) against the codling moth, *Cydia pomonella* (Linnaeus, 1758) (Lepidoptera: Tortricidae)

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Abstract

The codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae), is a key pest of apples in Tunisia and other countries around the world. Various management strategies have been applied to manage this pest including releases of parasitoids. *Trichogramma* wasps are known as efficient biocontrol agents tested against a large number of lepidopteran pests including *C. pomonella*. Two inundative releases of *Trichogramma cacoeciae* Marchal, at the rates of 25 000 and 50 000 parasitoids per ha, were performed when threshold was reached (2 to 3 adults per pheromone trap) in a commercial apple orchard, in 2018. Results indicated the occurrence of three flight peaks registered in the spring, summer and autumn of the growing season. The efficacy of the two release rates in reducing the infestation level of the population of the codling moth was proved herein. Average infestation level of fruits was significantly reduced after of the first release with reduction of about 27% for rate 1 and 22% for rate 2 compared to the untreated control (45%). Likewise, the average percentage of damaged apples was significantly reduced after the second release (23, 18.37 and 43.37 % respectively for the rate 1, rate 2 and the untreated control). The results showed the efficacy of *T. cacoeciae* parasitoids as efficient candidates to control *C. pomonella*.

1. INTRODUCTION

The codling moth *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) is a major insect pest of apples worldwide (Barnes, 1991). Other tree fruits including walnuts, pears, apricots, and plums was reported as susceptible hosts to this pest (Barnes, 1991; Wearing et al., 2001). This multivoltine species was able to achieve several generations per year as the case in Algeria (Dyhia and Ferroudja, 2016). Damages are caused by larvae which burrow into the fruit causing stings and deep entries making it unsuitable for consumption (Coutin, 1959; Barnes, 1991). Yield losses may exceed 80 % in case of heavy infestation (Barnes, 1991; Botto and Glaz, 2010). Management control strategies have relied mainly on broad-spectrum insecticides but their negative effects that may lead the development of resistant strains preclude their efficacy (Varela et al., 1993;

Knight et al., 1994; Sauphanor et al., 1994; Charmillot et al., 1999; 2007; Reyes et al., 2007). Biological control based on the use of predators and/or parasitoids are considered as effective and promising alternative to chemical insecticides (Hassan, 1993). Egg parasitoids belonging to genus *Trichogramma* (Hymenoptera: Trichogrammatidae) have already been tested against a wide range of lepidopteran pests including tortricidae pests thanks to their ubiquitous distribution, specificity and efficiency (Hassan, 1989, 1993; Smith, 1996; Mills, 2003, 2005; Cherif et al., 2021). Various *Trichogramma* species (e.g. *Trichogramma platneri* Nagarkatti, *T. evanescens* Westwood, *T. dendrolimi* Matsumura, *T. cacoeciae* Marchal and *T. pretiosum* Riley) are already known as efficient candidates to parasitize the codling moth worldwide (Bloem et al., 1998; Mills, 2003; Botto and Glaz, 2010; Bou-

Faour et al., 2016; Öztemiz et al., 2017; Sigsgaard et al., 2017). In Tunisia, the indigenous species *T. cacoeciae* is already commercialized for biological control of some lepidopteran species mainly *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), and *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae) (Cherif et al., 2013, 2019, 2023; Zougari et al., 2021).

Until recent dates, there is no data confirming the effectiveness of *T. cacoeciae* mass releases in reducing damages caused by *C. pomonella* in Tunisia. From this point of view, this study aimed to evaluate the effect of two release rates in controlling a *C. pomonella* population in apple orchard.

2. MATERIAL AND METHODS

2.1 *Trichogramma* mass rearing

Colonies of *T. cacoeciae* were maintained in the laboratory using alternative host eggs of the Mediterranean flour moth, *Ephesia kuehniella* Zeller (Lepidoptera: Pyralidae) (Cherif and Lebdi- Grissa, 2013). The later was reared in aerated boxes containing whole wheat flour. Irradiated *E. kuehniella* eggs were glued on cardboards (5 × 1 cm) and offered to newly emerged *T. cacoeciae* adults fed with a diluted honey solution. Afterwards, parasitized eggs were kept in glass tubes (10 × 1.5 cm) for inundative releases use. Both *Trichogramma* parasitoids and their host were maintained under controlled conditions (25 ± 1°C, 60 ± 10% RH and a photoperiod of 16: 8 (L: D)). The reared strain of *T. cacoeciae*, described above (Kio11 (EU882010)), was provided by the olive institute of Sfax (South of Tunisia).

2.2 Field releases

A private apple orchard, covering an area of one hectare, planted with 625 trees of Royal Gala variety and located in the Mornag region, (36° 40' 51" North, 10° 17' 25" East) was selected for this study. *Cydia pomonella* was monitored using two sex pheromone delta traps to survey the threshold (2 to 3 adults per pheromone trap) before *Trichogramma* releases. Traps were set up on late December, 2017 and checked from early January to late November 2018. A minimum distance of 50 m was kept between the 2 traps. Sex pheromone lures were renewed every 4 weeks.

Two releases of *T. cacoeciae* were applied on 20th July 2018 and 2nd August 2018, respectively when threshold was reached and a high number of the pest was found. During the release period and according to the Tunisian Meteorological

Institute, the mean temperature ranged from 30°C (July) to 26°C (August) and the mean relative humidity ranged from 49% (July) to 66% (August). Two rates (25 000 and 50 000 individuals /ha) were tested at each release. Three plots of ten apple trees each were fixed; two for test rates and one for control (no release); with a distance of more than fifteen meters between plots. No chemical treatment was applied when releasing *Trichogramma* parasitoids. Plates containing 40 and 80 parasitized eggs with *T. cacoeciae* and corresponding to the first and second rates respectively were released in each apple tree at a height of 1.5 m. A total of 400 and 800 plates were used for rate 1 and rate 2 at each release. To evaluate the efficacy of releases, 20 fruits were harvested at random from each tree of each plot devoting to the rate 1 (25 000 individuals/ha), rate 2 (50 000 individuals/ha) and the untreated control, one week after each release. Sampling was carried out on July 20th, July 26th, August 02th, August 09th, and August 16th, 2018. Visual inspections were carried out to distinguish infested apples, characterized by the presence of entry or exit holes, from clean ones. In some cases, especially when holes present in the calyx or stem end of apples are hard to detect, sampled apples were dissected.

To evaluate the emergence rate of *Trichogramma* wasps, five plates were retrieved at random from each treated plot devoted to the first and second rate one week after each release. The emergence rate was calculated following this formula (Huang et al., 2018):
Emergence rate (%) = (number of hatched *E. kuehniella* eggs / total number of black host eggs) × 100.

2.3 Statistical analyses

Statistical analyses were performed using the software SPSS 17 (SPSS Inc., 2012). Levene and Shapiro-wilk tests were performed to check data for their homoscedasticity and normality respectively. Data corresponding to the infestation level were subjected to arcsine square root transformation whenever necessary (Gomez and Gomez, 1984) and analyzed using repeated ANOVA. One-way ANOVA was performed to determine differences between the emergence rate and damages after the two releases. Means among treatments were separated with Duncan test at $P < 0.05$.

3. RESULTS

3.1 Codling moth male flight activity

Three flight peaks of *C. pomonella* were recorded respectively on 26th April, 12th July and 04th October 2018 throughout this study. The highest trap catches (25 males/trap/week) was registered in the summer during the second flight period in July (Fig. 1).

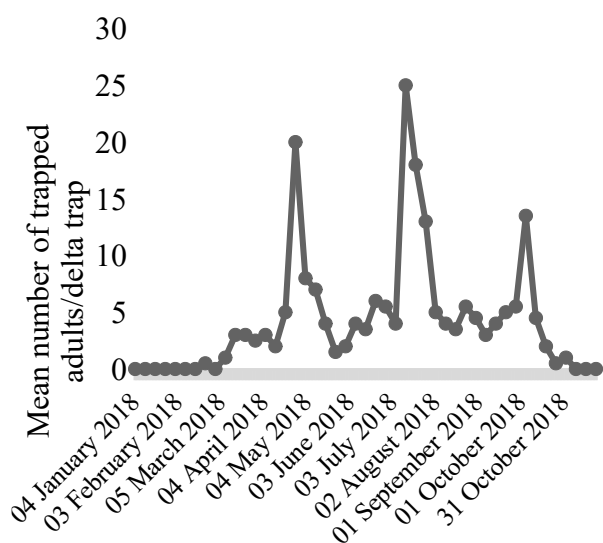


Fig. 1. Male flight activity of *C. pomonella* adults in apple orchard in Mornag region.

3.2 Trichogramma releases

Emergence rate exceeded 68% at the rate 2 in the 1st release (Table 1). Statistical analysis showed that there was non-significant difference between the two rates for the first ($F_{1, 9}=3.48$; $p=0.09$) and second release ($F_{1, 9}=0.52$; $p=0.49$), respectively.

Table 1. Emergence rates of released *T. cacoeciae* parasitoids in apple orchard in Mornag region

Releases	Emergence rate (%)	
	Rate 1	Rate 2
First release	52.50 ± 11.06 a	68.25 ± 12.73 a
Second release	60.00 ± 18.50 a	51.50 ± 14.50 a

Rate 1=25000 individuals /ha; Rate 2= 50000 individuals /ha; Mean ± Standard Error (SE) followed by the same letter do not differ significantly (Duncan test, $P < 0.05$); Lower letters following the means represent comparisons within the line.

The results indicated that *T. cacoeciae* releases significantly reduced the percentage of damaged apples caused by *C. pomonella* (Fig. 2).

Statistical analysis showed a significant difference between the two tested rates and the untreated control for the first release ($F_{2,29}=$

11.80, $P<0.001$) as well for the second one ($(F_{2,29}=13.69, p<0.001)$).

Non- significant difference was found between the two tested rates for the first and the second release (Fig. 2). The interaction between the two factors 'release rate and release time' had an impact on the infestation level caused by *C. pomonella* ($F=32.36$; $df=2$; $p<0.001$).

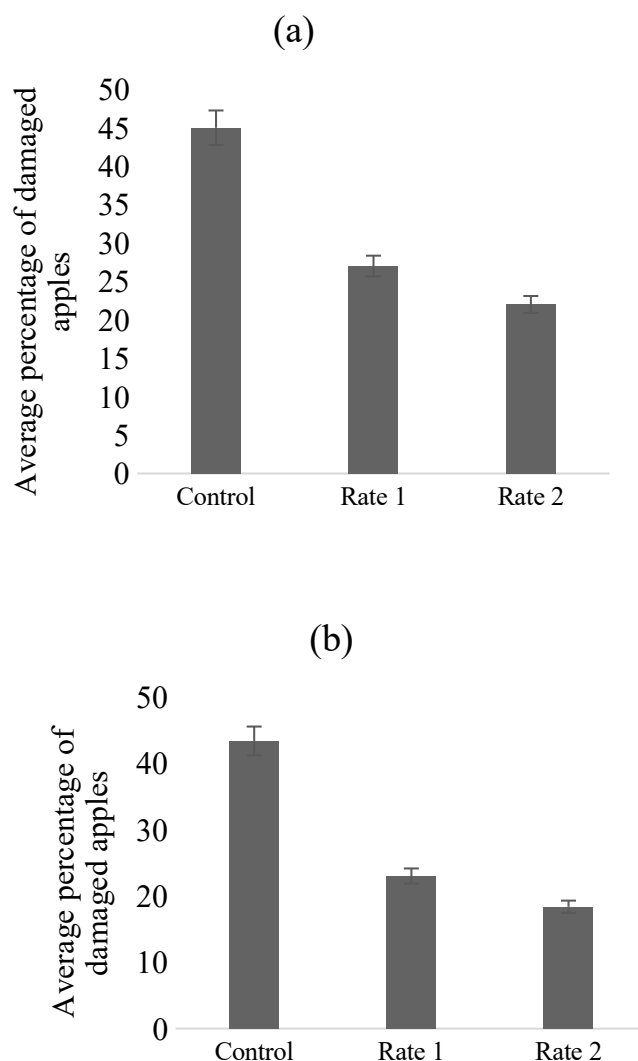


Fig. 2. Effect of 1st (a) and 2nd (b) *Trichogramma* releases on the number of damaged apples in apple orchard in Mornag region (Rate 1=25 000 individuals /ha; Rate 2= 50 000 individuals /ha)

4. DISCUSSION

Data revealed the occurrence of three flight peaks of the codling moth registered in the spring, summer and autumn. According to previous results, *C. pomonella* is a multivoltine species able to perform from one generation in cold areas compared to five in hotter ones (Audemard, 1991). In fact, flight activities of the codling moth depend on environmental conditions including summer length and climatic

changes (Stoeckli et al., 2012). Obtained results are similar to those obtained in Lleida, Spain by Bosch et al., (2016) who indicated that *C. pomonella* had three active periods of flight (May, June-July or July-August, August-September) in Spanish apple orchard during 2009 and 2010. In the same context, in Algeria, three flight periods of *C. pomonella* were recorded during April-May, June-July and August-September in apple orchard during 2009 and 2014 (Dyhia and Ferroudja, 2016).

Use of pheromone traps helps to apply effective management strategies when tolerance threshold is reached. The later varied from 2 to 3 males/trap/week in Catalonia apple orchards for example (González, 2007). Here, two *Trichogramma* release rates (25 000 and 50 000 individuals/ha) using the autochthone strain *T. cacoeciae* were applied against the codling moth during the second flight period corresponding to the summer activity when insect population is more abundant.

Emergence rate of *Trichogramma* wasps recorded herein exceeded 68%. A high emergence rate was already recorded for this species when released against the tomato leafminer *Tuta absoluta* (up to 81%) under tomato open field crop in northeastern Tunisia in 2016 (Cherif et al., 2019) or against the carob moth *Ectomyelois ceratoniae* (up to 94.8%) in pomegranate and date palm trees in south-west of Tunisia in 2016 (Zougari et al., 2021).

The present results indicated that *C. pomonella* damages were significantly decreased after the two inundative releases than the untreated control with non-significant difference obtained between the two release rates. Previous studies highlighted the efficacy of *T. cacoeciae* when released with high rates against the codling moth in apple orchards. In this context, a significant reduction in the infestation rate up to 72.69% was recorded in Sweida, Syrian apple orchards (var. Golden delicious) after a weekly release of 250 000 *T. cacoeciae* /ha during the growing season until the harvest in 2012-2013 and 2014 (Bou-Faour et al., 2016). However, Almatni et al., (2002) demonstrated that a release rate of 150 parasitoids/ tree was not efficient in reducing the infestation level during the second generation of *C. pomonella* in Syrian apple orchard (var. Golden delicious) in 2001.

Releases of *Trichogramma* parasitoids in apple orchards have given promising results when combined with other control strategies as mating disruption (Sigsgaard et al., 2017), sterile

insect technique (Cossetine and Jensen, 2000) or *Bacillus thuringiensis* application (Öztemiz et al. 2017). Previously, it has been shown that fruit damages were decreased up to 3.3% after releases, every two or three weeks, of 1000 individuals per tree of *T. evanescens* and *T. cacoeciae* alones or mixed in Denmark where mating disruption regime was applied in 2013 (Sigsgaard et al., 2017).

5. CONCLUSION

The present results confirmed that the codling moth is able to perform up to three flight periods in Tunisian apple orchard. Monitoring with pheromone traps can not only provide information about the occurrence of the pest in the field but also may help farmers to choose the most efficient management strategy. Obtained data showed that *T. cacoeciae* could be a promising candidate for biological control of *C. pomonella* especially when released at reached threshold. The two tested rates were judged to be efficient in reducing damages (up to 18.37%) caused by this pest in case of low infestation level. However, further studies are needed to optimise biological control of *C. pomonella* with *Trichogramma* wasps taking into consideration major factors including high costs compared to insecticides sprays.

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