

Reduction in Carob Moth *Ectomyelois ceratoniae* (Lepidoptera, Pyralidae) populations by means of mass trapping pheromone and *Trichogrammacacoecia* (Hymenoptera: Trichogrammatidae) releases in Tunisian oases

S. BEN CHAABAN¹, K. MAHJOUBI¹

¹ Regional Research Centre of Oasis Agriculture, Degache, Tunisia

*Corresponding author: samah_bchaaban@yahoo.fr

Abstract – In Tunisia, the carob moth, *Ectomyelois ceratoniae* is considered as the main pest of dates and pomegranates. The purpose of this study is to evaluate the efficacy of the mass trapping pheromone combined with *Trichogramma cacoeciae* release to control the the carob moth populations. Field experiments were conducted in 2018 in three oases located in the south of Tunisia. The efficacy of the treatment was evaluated by assessing male captures in traps and percentage of fruit infestation. Results showed that the highest numbers of carob moth adults were captured in untreated oases compared with the control plots. The treatment provided 72.9 and 52.8% reduction of insect captures respectively on date palm and on pomegranate in Tayfout oasis. In Hazoua and Rjim Maatoug oases the capture reductions were 78.6% and 46% respectively. Dates infestation was highest in the control plots (15%, 10% and 7.2 %) respectively in Tayfout, Rjim Maatoug and Hazoua oases compared with treated plots (3.2, 3 and 2.3%). Regarding pomegranate, rate of fruit damage was significantly lower in the treated oasis compared to the control oasis.

Keywords: *Ectomyelois ceratoniae*, mass trapping, *Trichogramma cacoeciae*, date palm, pomegranate

1. Introduction

The carob moth, *Ectomyelois ceratoniae* is the key pest of many crops worldwide. It induced great damage to the crops annually and is of concern to growers as few insecticides are available for controlling this pest (Vetter et al. 1997). In Tunisia, *E. ceratoniae* is the major insect pest of dates, pomegranate and several other host plants in both field and storage (Dhouibi 1989; Jarraya and Vinson 1980). In fact, In Tunisia, the annual infestation rates can be anything from 20% in dates to 80% in pomegranates (Mediouni & Dhouibi 2007). Despite coverture dates with filets, the pest causes significant damage and reduces marketability of dates in Tunisia. This pest causes great economic losses and yearly infestation rates range from 20% in dates to 80% in pomegranate in Tunisia (Dhouibi 1982, 1992). However, insecticides seem to be inefficient due to its endophytic behaviour and the dangling position of the fruit on the pomegranate tree (Dhouibi et al. 2000).

Alternative control strategies, such as mating disruption with synthetic pheromone (Vetter et al. 2006; Dhouibi et al. 2017) conservation of natural enemies (Basirat et Mehrnejad 2005), mass trapping (Dhouibi et al. 2016), Attract and Kill method (Dhouibi et al. 2016) are being studied for their potential role in an integrated pest management program for *E. ceratoniae*. The augmentative release of mass-reared *Trichogramma* egg parasitoids has been identified as a promising method to reduce both eggs hatching and subsequent damage due to larval feeding *E. ceratoniae* (Lebdi-Grissa and Ben Ayed 2005; Dhouibi et al. 2016 Dhouibi and Jammazi 1993; Khouldia et al. 1996).

The present study investigates the effectiveness of mass trapping combined with trichogramma release for controlling the carob moth *Ectomyelois ceratoniae* population in south Tunisia oases in an Integrated Pest Management approach (IPM).

2. Material and methods

2.1. Experimental fields

Experiments were conducted in three oases, with an area of 1 hectare each, in two governorates located in the south of Tunisia: Hazoua (governorate of Tozeur) date palms Deglet Noor variety; Rejim Maatoug



(governorate of Kebili) date palms Deglet Noor variety and Blidet (governorate of Kebili) where pomegranate trees and palm dates were planted.

2.2. Treatments

Mass trapping and release of *T. cacoecae* were tested at the same time on date palms and pomegranate trees in Blidet oasis. Hazoua and Rejim Maatoug oases received a combination mass trapping and release of *T. cacoecae* on date palms only. In addition, at each oasis, one orchard (1 ha) was used as control plots remained untreated (no release of *T. cacoecae*, no mass trapping). More details are reported in Table 1.

The experimental design consisted of fifteen white delta traps baited with the sex pheromone ((Z,E)-7,9,11,-dodecatrien-1-ol formate) suspended on dates palm trees in grapes, and hung to pomegranate trees at a height of 1.5 m in Blidet oasis. The traps were checked fortnightly and the trapped moths were counted and removed from the traps. Population monitoring had been carried out from May 2016 and continued until the end of this experiment. The pheromone baits were also changed with fresh ones in every four weeks. The indigenous strain of *T. cacoecae*, was previously collected from the Tozeur governorate, Tunisia. Rearing of the parasitoid was performed on eggs of *E. kuehniella* (Lepidoptera: Pyralidae), which were routinely reared in the laboratory at 25±1 °C, 60±10% relative humidity, and a 16:8 photoperiod.

Table 1. Characteristic of the different Oases

Plot	plantation	Treatment	Dose of the used product
Tayfout	Date palms pomegranate trees	Mass trapping and <i>Trichogramma cacoeciae</i> release on date palm and pomegranate trees	-15 traps/ha on date palm - 6 traps/ha on pomegranate trees -1 <i>Trichogramma card</i> (300eggs)/ pomegranate tree on 17/5, 7/6, 12/7, 9/8, 6/9 -10 <i>Trichogramma card</i> (300eggs)/ date palm tree on 6/9
Hezoua	Date palms	untreated	
	pomegranate trees	untreated	
	date palm	untreated	
RejimMaatoug	date palm	Mass trapping and <i>Trichogrammacacociae</i> release on date palm	15 traps/ha 10 <i>Trichogramma card</i> (300eggs)/ date palm tree on 5/9
	date palm	untreated	
	date palm	Mass trapping and <i>Trichogrammacacociae</i> release on date palm	15 traps/ha on date palm 10 <i>Trichogramma card</i> (300eggs)/ date palm tree on 17/5
	date palm	untreated	

2.3. Estimation of field infestation

At harvest, ten palm date were selected from each orchard were tagged for sampling. These trees were chosen at random to assure coverage of the orchard, 2 trees located in the North, South, West, East and in the middle of oases. Samples of 25 abscised fruit was collected from the north, south, east, west and middle of the trees at an intermediate height, totalling 100 fruits/tree. The fruits were dissected in the laboratory to evaluate the infestation rates of *E. ceratoniae*.

The percentages of abscised fruit per date palm or pomegranate with infestation were compared between treatment and control by an analysis of variance (ANOVA). The capture inhibition was calculated as (1-average plot catch/average control plot catch).

3. Results

3.1. Tayfout oasis

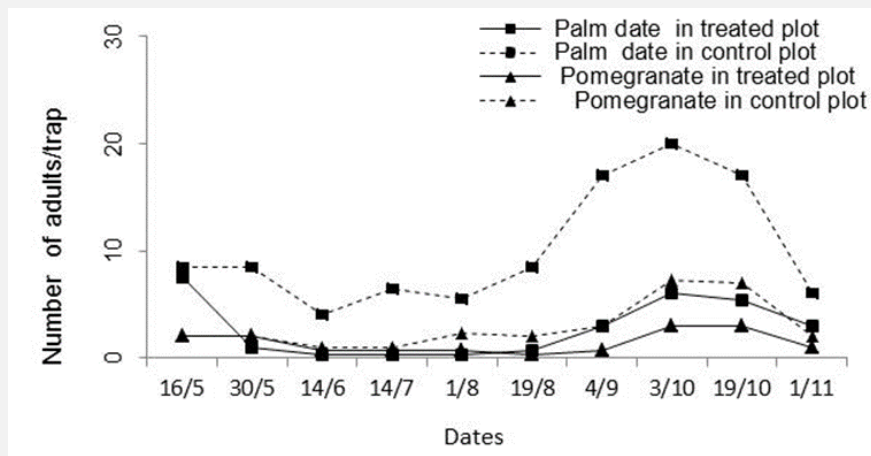


Figure 1. Evolution of the mean number of *E. ceratoniae* females captured fortnightly per trap on date palm and pomegranate trees in Tayfout oasis.

Catches data from monitoring traps on date palm and pomegranate were collected fortnightly in Tayfout treated plot and were compared to the control (Figure 1). Pheromone trap fixed on date palm and pomegranate showed a presence of *E. ceratoniae* population from May 16 until the first week of November, but seasonally fluctuating. Prior to the establishment of the experiment, the adult *E. ceratoniae* population on date palm densities were 7.5 in treated plot and 8.5 in the control plot, corresponding to the overwintering populations.

The population on date palm and on pomegranate trees increased mainly in September and October. The highest of insect numbers caught on date palm and pomegranate, were recorded at 3/10. The results showed that the traps captured 421 and 121 male moths respectively on date and on pomegranate during a period of survey. Regarding to the mean carob moth captures, differences were observed between the treated and the control orchards regarding date palm ($F(1, 18) = 13.9, P < 0.05$). Considering pomegranate there were no significant differences among the mean of captures between treatment and control ($F(1, 18) = 3.9, P = 0.063$).

The efficacy of the mass trapping combined with *Trichogramma* release was also performed by the estimation of the capture inhibition for treatments provided 72.9 and 52.8 reduction respectively on date palm and on pomegranate. Treatment Efficacy was also executed at harvest, we evaluated the effect of different treatments on the evolution of the infestation level by examining respectively samples of abscised date fruits and pomegranate fruits (Figure 4) the infestation level by *E. ceratoniae* decreased to reach 3.2% compared to 15% in the control plot and 4% compared to 20% (Figure 4).

3.2. Hazoua oasis

Pheromone trapping took place on date palms between May 10 and October 25, on the Hazoua oasis. The seasonal activity of the pest varied during the sampling period in treated and control oases (Figure 2). The captures increased steadily, peaking on 28 June, and then falling reaching a second peak on 17 August captures increased at that point while it decreased from the end of August to October.

The males captured in the pheromone traps indicate that mass trapping combined with *cacoeciae* release significantly lowered the rate of infestation of *E. ceratoniae* relative to the control plot ($F(1, 20) = 5.147, P < 0.05$). The total of trapped *E. ceratoniae* was 98 male in the treated plot, the corresponding trap inhibition was 78.6%.

In addition, this effective controlling *E. ceratoniae* population in the treated field reduced significantly the rate of infested date fruits (2.3) compared to untreated plot (7.2%) (Figure 4).

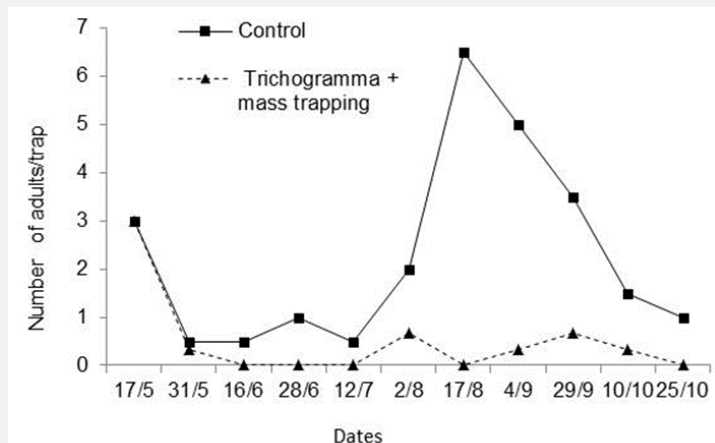


Figure 2. Evolution of the mean number of *E. ceratoniae* males captured fortnightly per trap on date palm in Hazoua oasis.

3.3. RjimMaatoug oasis

From May until harvest of dates, we evaluated the effect the mass trapping and trichogramma release on the evolution of the carob moth populations in RjimMaatoug oasis.

Pheromone trap catches showed an always present, but seasonally fluctuating population from May 20 until October 28 (Figure 3). The infestation rate in control and treated plots increased gradually until 4 August, and reached a maximum 4 and 1,33 of for the control and treated plot. The treatment lowered the infestation. Nevertheless, no statistical differences were observed between treated and control plots ($F(1, 20) = 3.01, P=0.098$), and the corresponding trap inhibition was 46 %. Likewise, the percentage of infested date fruits obtained in the control plot (Figure 4) was approximately 3 times higher than that of the treated plot with respectively 10 and 3 %.

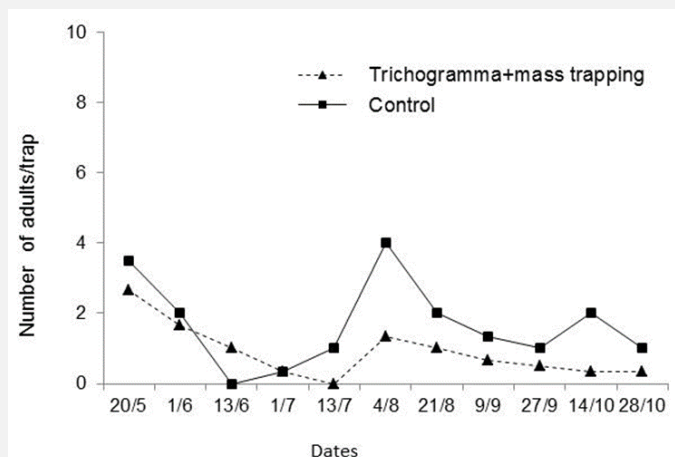


Figure 3. Evolution of the mean number of *E. ceratoniae* males captured fortnightly per trap on date palm in RjimMaatoug oasis.

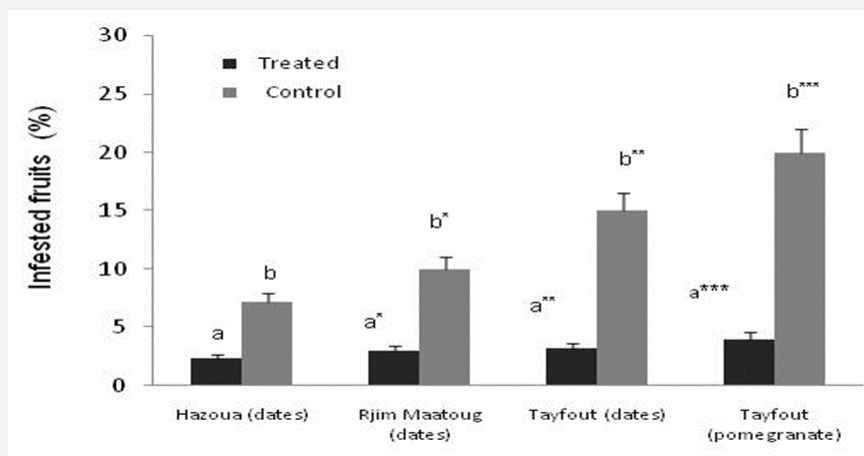


Figure 4. Percentage of fruit damage on dates and pomegranate in the treated and control plots in Hazoua, Rjim Maatoug and Tayfout oases .

4. Discussion

Throughout the entire survey period, the major flight period of carob moth was occurred from may through October in all studied plots. The increases recorded in the spring period, especially in May and June, are to be considered as a consequence of the emergence of the pest population that overwinters as mature larvae or pupae. The main annual peaks occurred during August, September or October months. This peak coincides with pomegranate and dates ripening period. Whereas the population remained at low levels from November, with few trap catches found.

The highest number of male adults per pheromone-baited trap was different according to the locality of oasis. In fact, in the different oases, the most important peak of captured males was detected at 3/10 in Tayfout (20 males/trap on date palm and 7 males/trap on pomegranate), at 1/8 in Hazoua (7 males/trap) and at 4/8 in Rjim Maatoug. Previous studies demonstrated that the generation of the carob moth in oases can be differed according to the locality of oasis type of vegetation which it occurs and by temperature (Demirel 2016, Dhouibi 2017). The development cycle of the carob moth takes 48 days at 20°C, 30 days at 25°C and 23 days at 30°C (Cox 1976).

The main purpose of our study was to reduce, *E. ceratoniae*, improving the mass trapping technique and *Trichogramma cacoecia* release. The current study demonstrated that the pressure exert by *E. ceratoniae* and its damage on date palm and pomegranate significantly decreases by the adopted treatment. Reduction in moth catches also corresponded well with the reduction in fruit damage on dates and pomegranate fruits.

Nowadays, pheromones and other semiochemicals are applied to monitor and control pests in millions of hectares (Senthil-Nathan 2015) and the diversity of applications of pheromones is increasing (Copping 1998). Pheromone attraction between males and females in Lepidoptera is common and well known (Jurenka 2003). For that the seasonal flight period of *E. ceratoniae* was established by the use of pheromone traps in many countries (Demirel et al. 2016; Norouzi et al. 2008; Lebdi-Grissa and Ben Ayed 2005). Moreover, Pheromones are used in mass trapping for control of insect pests has been suggested for a number of Lepidoptera pests (Tan et al. 1996). Mass trapping to target lepidopteran pests consists of deploying traps baited with sex pheromone to attract and catch males before they locate and mate with females (El-Sayed et al. 2006). In this regard, Mediouni (2005) found that the control of *E. ceratoniae* trapping with pheromones at 12 traps per hectare in pomegranate orchard provided decreased to 4.5% in 2004 compared to 28% in 2001 in the control plot. According to Dhouibi et al. (2016), reported that the use of 10 delta traps per hectare in pomegranate orchard was effective in controlling of the carob moth. In fact, after 16 days of the treatment, the reduction of the infestation rate reached 100%. However, no study has mass trapping method against carob moth on dates.

Egg parasitoids of the genus *Trichogramma* have been used successfully as inundative biological control agents against the eggs of a large number of important lepidopteran pests and are the most widely used natural enemies in biological control worldwide (Li 1994; Smith 1994; Hassan 1993). It is considered as the most described parasitoid genus, and consists of approximately 180 species (Rohi et Pintureau 2003). They are the most widely used biological control agents, partly due to their easy mass rearing and their

potential to kill many important crop pests before they leave the egg and cause feeding damage (Hassan1985; Ulrichs et Mewis2004). Different native species of *Trichogramma* have been evaluated for controlling the carob moth pomegranate orchards, (Moezipour 2006; Sayed 2015; Ksentini et al. 2010). In Tunisia *T. cacoecae* has great potential as a biological control agent, particularly because it's a native *Trichogramma* matching with Tunisian ecological conditions (Lebdi-Grissa et Ben Ayed 2005; Dhouibi et al. 2016; Dhouibi et Jammazi 1993; Khoualdia et al. 1996). Doses used in fields vary from one study to another. For example, for a single release of 25000 *Trichogramma*/ha (3000 eggs/Pomegranate tree) significantly lowered the level of infestation. In fact, the infestation in the plot selected for these releases decreased from 30% (control plot) to 12%(Dhouibi2016).Moreover, Lebdi et Ben Ayed (2006), founded that the release of *Trichogramma cacoeciae*, in Tastour on pomegranates orchard reduced the infestation percentage from 13.2% to 2.4%.For example inundative release of a total of 157000 eggs/ha on date palms reduced the infestation level from %16.5in the control plot to %3.5in the treated plot (khoualdia 2006).

5. Conclusion

The mass-trapping technique combined with *T. cacoecae* release included in an IPM program that includes improved date orchard sanitation techniques showed some promise for suppressing low-density populations of *E. ceratoniae*. The study one of the first to evaluate *Trichogramma* release under a mass trapping regime. We need improvement of mass trapping and *T. cacoecae* releases on a long-term study, more than one year. We need mainly to further study the timing and number of releases and *T. cacoecae* doses for the use of this control agent over large areas.

Acknowledgements

We would like to thank reviewers for their comments and suggestions, helpful to improve the first draft of the manuscript. We acknowledge financial support from the VacpaBoujbel. The authors wish to thank staff VacpaBoujbel for his assistance during field surveys include. and the farmers in and for permission to use their crop fields

6. References

- Basirat M, Mehrnejad M** (2005) Letter Iran Entomol Society. 24 : 19-34
- Copping LG** (1998) The BioPesticide Manual. British Crop Protection Council, Surrey UK.
- Cox PD** (1976) The influence of temperature and humidity on the life-cycle of *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae). J Stored Prod Res. 1: 2 111-1 17
- Demirel N** (2016) A study on occurrence and population trends of the carob moth, *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae) in pomegranate orchards by using pheromone traps. Entomol ApplSci Lett. 3 (1):26-31
- Dhouibi MH** (1982) Bioécologie d'*Ectomyelois ceratoniae* Zeller (Lepidoptera:Pyralidae). Annales de l'INAT. 55(4) p34.
- Dhouibi MH** (1989) Biologie et écologie d'*Ectomyelois ceratoniae* Zeller. (Lepidoptera: Pyralidae) dans deux biotopes différents au sud de la Tunisie et recherches de méthodes alternatives de lutte. Doctorat d'état en sciences naturelles, Université Pierre et Marie Curie, Paris VI, 176 pp
- Dhouibi MH** (1992) Effet de la Bactospeine XLV sur la pyrale des dattes, *Ectomyelois ceratoniae* Zell. (Lepidoptera: Pyralidae). Med Fac LandbouwwUniv Gent 57 : 505-514
- Dhouibi MH, Jammazi A** (1993) Lutte biologique contre la pyrale des caroubes par *Habrobracon hebetor* en verger de grenadier. Med FacLandbouwwUnivGent 57(2b) : 427-436
- Dhouibi MH, Hawlitsky N, Zaaaraoui H, Krisaane T, Cheikh T, Cherni M, Ben Moussa I** (2000) Biological control against the carob moth *Ectomyelois ceratoniae* in Oasis and in packing houses in Tunisia. Area-wide control of fruit flies and other insect pests, ed. K.H. Tan.PenerbitUniversitiSains Malaysia, Penang : 229-236

- Dhouibi MH, Hawari W, Touil S, Zouba A, Ben Hamida F (2017)** Biocontrol of the Carob Moth *Ectomyelois ceratoniae* (Lepidoptera, Pyralidae) in Two Oases in the South of Tunisia using Mating Disruption with SPLAT EC O. *Int J Agri Inno Res* 5(5): 825-830
- Dhouibi MH, Hermi H, Soudani D, Thlibi H (2016)** Biocontrol of the carob moth *Ectomyelois ceratoniae* in pomegranate and citrus orchards in Tunisia. *Int J Agri Inno Res* 4(5): 849-856
- El-Sayed AM, Suckling DM, Wearing CH & Byers JA (2006)** Potential of mass trapping for long-term pest management and eradication of invasive species. *J Econ Entomol*: 99: 1550-1564
- Hassan SA (1993)** The mass rearing and utilization of *Trichogramma* to control lepidopterous pests Achievements and outlook. *PesticSci*37: 387-391
- Jarraya A, Vinson G (1980)** Contribution à l'étude de l'entomofaune du pistachier. Observations biologiques et écologiques sur *Ectomyelois ceratoniae* Zeller (Lépidoptère: Pyralidae). *Annales INRAT*. 53: 1- 42
- Jurenka R (2003)** Insect pheromone biochemistry and molecular biology, Oxford: Elsevier Academic Press, chapter Biochemistry of female moth sex pheromonespp. 53-80.
- Khoualdia O, R'houma A, Marro JP (1996)** Utilisation de *Trichogrammacacoeciae* Marchal (Hymenoptera, Trichogrammatidae) contre la pyrale des dattes. *Annales de l'INRAT*69 :197-205
- Khoualdia O, Pizzol J, Ferran A (2008)** Capacité de dispersion vertical chez *Trichogramma cacoeciae* Marchal (Hym: Trichogrammatidae) dans les palmeraies du sud Tunisien. 8th International Conference on Pests in Agriculture, Montpellier, 8p.
- Ksentini I, Monje JC, Jardak T, ZEGHAL N (2010)** Naturally occurring egg parasitoids of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) in a pomegranate orchard in Tunisia *Entomological Science* 13, 99–106
- Lebdi-Grissa K, Ben Ayed N (2005)** Lutte biologique contre *Ectomyelois ceratoniae* sur grenadier par des lâchers de *Trichogramma cacoeciae*. AFPP 7ème Conférence Internationale sur les Ravageurs en Agriculture, Montpellier, 7p
- Li LY (1994)** Worldwide use of *Trichogramma* for biological control on different crops: A survey, pp. 37-53 In E. Wajnberg and S. A. Hassan [eds.], *Biological Control with Egg Parasitoids*. CAB International, Wallingford
- Mediouni J., 2005.** Lute génétique contre la pyrale des caroubes *Ectomeylois ceratoniae* Zeller 1881 (Lepidoptères: Pyralidae) par le biais de la technologie des insectes stériles. Thèses de Doctorat en sciences agronomiques, INAT, 153 p
- Mediouni J, Dhouibi, MH (2007)** Mass-rearing and field performance of irradiated Carob moth *Ectomyelois ceratoniae* in Tunisia. *Area-Wide Control of Insect Pests*. 265-273.
- Moezipour M (2006)** Effect of different temperature and humidity treatments on some biological parameters of two *Trichogramma brassicae* Bezd. populations, collected from pomegranate orchards of Yazd and Saveh. 161 pp. M.Sc. Thesis, Isfahan University of Technology, Iran
- Norouzi A, Asghar A, Talebi AA, Fathipour Y (2008)** Development and demographic parameters of the carob moth *Apomyelois ceratoniae* on four diet regimes. *Bull Insectol* 61: 291-297
- Rohi L, Pintureau B (2003)** Are *Trichogramma bourarachae* and the *perkinsi* species group really distinct from *Trichogramma buesi* and the *pinto*i group, respectively? *Journal of Applied Entomology* 127: 265-268
- Sayed SM, Elsayed G, Mahmoud SF, Elzahrany OM (2015)** Efficacy of *Bacillus thuringiensis* and indigenous *Trichogrammatürkistanica* for controlling lepidopterous pests on Taify pomegranate fruits. *Afr Entomol*23(2):443-450
- Senthil-Nathan S (2015)** A review of biopesticides and their mode of action against insect pests, in: *Environmental Sustainability*, Springer, India, 49-63

- Smith SM(1994)** Methods and timing of releases of *Trichogramma* to control lepidopterous pest. In: E. Wajnberg and S.A. Hassan, eds. Biological control with egg parasitoids. Wallingford: CAB International : 113-144
- Tan ZX, Gries R, Gries G, Lin GQ, Pu GQ, Slessor KN, LiJ (1996)** Sex pheromone components of mulberry looper, *Hemerophilaartilineata* Butler (Lepidoptera: Geometridae). J ChemEcol22: 2263-2271
- Ulrichs C, Mewis I (2004)** Evaluation of the efficacy of *Trichogramma evanescens* Westwood (Hym. Trichogrammatidae) inundative releases for the control of *Maruca vitrata* F. (Lep., Pyralidae). J Appl Entomol 128: 426-43
- Vetter RS, Tatevossian S, Baker TC (1997)** Reproductive behavior of the female carob moth, (Lepidoptera: Pyralidae). Pan-Pacific Entomologist 73: 28-35
- Vetter RS, Millar JG, Vickers NJ, Baker TC (2006)** Mating disruption of *carob moth*, *Ectomyelois Ceratoniae*, with a sex pheromone analog. South West Entomol 31(1): 33-47