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# Side-effects of some insecticides used in citrus cultivation on the egg parasitoid *Trichogramma cacoeciae* Marchal immature stages

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**Abstract**
In order to monitor *Ectomyelois ceratoniae* Zeller (Lepidoptera: pyralidae) in citrus orchards, an integrated pest management (IPM) approach is needed. However, this control method implies the releases of natural enemies such as *Trichogramma* species besides to pesticides spraying which represents a potential risk for the useful fauna. Thus, the aim of this study is to investigate the side effects of six insecticides commonly used in citrus fields on the *Trichogramma cacoeciae* Marchal immature development stages.
In the National Agronomic Institute of Tunisia, laboratory studies were carried out in May 2016. Two biological (Spinosad and *Bacillus thuringiensis*) and four chemical (Abamectin, Dimethoate, Malathion and Chlorpyriphos-ethyl) treatments were tested under laboratory conditions (25±1°C; 60±10% RH; L/D: 16/8) on *T. cacoeciae* immature development stages (larvae, prepupae and pupae).
According to the International Organization of Biological Control (IOBC), the results obtained confirmed that Spinosad treatment was more toxic when applied on the larvae stage compared to prepuape and pupae stages. Whereas, Abamectin, Dimethoate and *Bacillus thuringiensis* treatments have a slightly harmful effect on *T. cacoeciae* independently of the development stage. However the Malathion effect, varied according to the immature stage on which it was applied on. Concerning Chlorpyrifos-ethyl, this chemical treatment was harmful towards all the development stages of the tested *Trichogramma* species. These findings will allow determining the proper time of treatments spraying to preserve the useful fauna.

**Keywords:** Citrus cultivation, *Ectomyelois ceratoniae*, egg parasitoid, IPM, insecticides, toxicity.

## 1. Introduction
In Tunisia, the citrus cultivation plays an important role in the agricultural sector. Unfortunately, this cultivation faces many problems, especially, the attacks of a large range of insects among them, *Ectomyelois ceratoniae* Zeller 1881 (Lepidoptera: pyralidae) commonly known as the date moth or the carob moth [8]. This pest which is particularly polyphageous, infests mainly the date palm *Phoenix dactylifera* L and the pomegranate fruits *Punica granatum* L in Tunisian orchards, where looses could reach 18 and 90% respectively [4, 2]. In order to monitor this moth, several control strategies have been adopted. However, biological control thanks to releases of its hymenopterans natural parasitoids especially those belonging to the Braconidae family such as: *Habrobracon hebetor* Say and *Phanerotoma ocuralis* Kohl [5, 10] besides to the Trichogrammatidae family such as *Trichogramma embryophagum* Hartig [6, 16], proved to be the most satisfactory. Indeed, egg parasitoids of the genus Trichogramma are the natural enemies of various lepidopteran pests, the most widespread and widely used as biological control agents in the world [14]. The efficacy of these wasps remains in their ability to eliminate their host's pest by laying in their eggs and developing at their expense [9].
Of particular interest, an indigenous strain *Trichogramma cacoeciae* Marchal (Hymenoptera: Trichogrammatiadae) has been reported to be efficient in regulating populations of *E.ceratoniae* in Tunisian palm date and pomegranate orchards [11, 13].
In order to control this pyralid in citrus orchards, an integrated pest management (IPM) program is needed. However, the use of pesticides could affect the useful fauna [7]. Therefore, detailed knowledge of the treatments effects on the natural enemies' most susceptible stages will help to determine the proper time of spraying to avoid them [19]. Accordingly, the purpose of this study is to evaluate the side- effects of six commonly used insecticides in citrus cultivation on the immature development stages of *Trichogramma cacoeciae* Marchal biologicl agent control of *Ectomyelois ceratoniae* Zeller, under laboratory conditions.

## 2. Materials and Methods

### 2.1 Egg parasitoid rearing

*Trichogramma cacoeciae* Marchal populations used for the experiments were reared on the host egg substitute *Ephestia kuehniella* Zeller under laboratory conditions (25±1°C; 60±10% RH; L/D: 16/8) in the Entomology-Acarology laboratory of the National Agronomic Institute of Tunisia.

### 2.2 Treatments tested

Two bio-pesticides: Spinosad (60cc $hl^{-1}$) and *Bacillus thuringiensis* Berliner (250g $hl^{-1}$) besides to four chemical insecticides: Abamectin (15cc $hl^{-1}$), Dimethoate (100cc $hl^{-1}$), Malathion (200cc $hl^{-1}$) and Chlorpyrifos-ethyl (150cc $hl^{-1}$) were used in the experiments. The concentration of each insecticide solution reflected the recommended citrus field rates. Distilled water was considered as control.

### 2.3 Experimental protocol

The study was conducted in May 2016 and the experimental protocol was carried out according to [12].

### 2.4 Determination of the treatments effects

The reduction in emergence was calculated, using the Abbott formula [1].

$$E\,(\%) = \frac{C-T}{C} \times 100$$

(C: emerged adults in control, T: emerged adults in Treatments)

The values E calculated for the insecticide treatments were classified according to the International Organization of Biological Control (IOBC): class 1—harmless (E<30%), class 2— slightly harmful (30%<E<79%), class 3—moderately harmful (80%<E<99%) and class 4—harmful (E>99%) [12].

### 2.5 Statistical analyses

The statistical analyses were performed using the statistical software program SPSS (Inc, 2007), Version (0.16). Data related to the Reduction in Emergence rates were subjected to one-way ANOVA. Duncan's multiple range tests at $P$=0.05 was used to separate Means of Emergence Rates (SPSS Inc, 2007).

## 3. Results and Discussion

Results from the present experiment are shown in Table 1. All the applied treatments reduced the emergence of *T. cacoeciae* parasitoids regardless of the development immature stage.

This study showed that Spinosad was more toxic when applied on the larvae stage (Class 4: harmful) compared to prepuape and pupae stages (Class 3: moderately harmful). These results did not corroborate with those obtained by [12] who indicated that this treatment was moderately harmful (Class 3) to both larvae and pupae stages compared to the prepupae stage (Class 2: slightly harmful). Besides, [15] confirmed the slightly harmful effect of this treatment when applied on *Trichogramma atopovirilia* Oatman & Platner from treated eggs of *Anagasta kuehniella* Zeller. Moreover, [18] indicated that this biopesticide had slightly harmful effect on the immature stages of *Trichogramma chilonis* Ishii reared on *Helicoverpa armigera* Hübner eggs.

Abamectin, Dimethoate and *Bacillus thuringiensis* were slightly harmful (Class 2) to *T. cacoeciae* independently of the development stage on which they were applied. In fact, this result was confirmed on Abamectin (sprayed on the immature stages of *Trichogramma pretiosum* Riley within host eggs *Anagasta kuehniella* (Zeller)) applied on the larvae stage. But, when applied on the pupae stage, this chemical treatment was moderately harmful (Class 3) [17].

As for Dimethoate, it was proved moderately harmful (Class 3) when applied on the *T.cacoeciae* pupae stage [20].

In contrary, [12] showed that *Bacillus thuringiensis* applied at the doses of 70 and 100 gr $hl^{-1}$ was harmless (Class 1) to all the *T. cacoeciae* immature stages.

Our research revealed that Malathion treatment was harmful to the prepupal stage, slightly harmful to the pupal stage and harmless to the larvae stage, however [20] showed that this treatment was harmless to the pupal stage.

The highest reduction in the emergence success of *T. cacoeciae* was caused by chlorpyrifos-ethyl. Indeed; this chemical treatment was harmful to all the immature stages (Class 4). Other studies confirmed that chlorpyrifos was highly toxic on *T. cacoeciae* adults [3], but when applied on *T. atopovirilia* immature development stages, this insecticide was harmless [15].

**Table 1:** Variation in Emergence Rate mean average (±SE) [1] , Reduction in Emergence (%)[2],Toxicity Class [3] of *Trichogramma cacoeciae* treated with different insecticide solutions during immature life stages (25±1°C; 60±10% RH; L/D: 16/8).

| Treatment (applied dose) | Immature development stage treated | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Larvae[1]** | **E(%) [2]** | **Class [3]** | **Prepupae [1]** | **E(%) [2]** | **Class [3]** | **Pupae [1]** | **E(%) [2]** | **Class [3]** | |
| Spinosad (60ml $hl^{-1}$) | 0 Aa | 100 | 4 | 0.155±0.066 Bb | 81.54 | 3 | 0.011±0.006 Aa | 98.69 | 3 | P=0.000, F=30.664, df=17 |
| Abamectin (15ml $hl^{-1}$) | 0.639±0.096 Ca | 33.85 | 2 | 0.530±0.122 Ca | 36.9 | 2 | 0.531±0.324 Ba | 36.78 | 2 | P=0.619, F=0.496, df=17 |
| Dimethoate (100 ml $hl^{-1}$) | 0.220±0.129 Ba | 77.22 | 2 | 0.544±0.135 Cb | 35.23 | 2 | 0.536±0.025 Bb | 36.19 | 2 | P=0.000, F=17.218, df=17 |
| *Bacillus thuringiensis* (250g $hl^{-1}$) | 0.656±0.204 Cb | 32.09 | 2 | 0.980±0.008 Ec | -0.16 | 1 | 0.336±0.160 Ba | 60 | 2 | P=0.000, F=27.587, df=17 |
| Malathion (200 ml $hl^{-1}$) | 0.706±0.087 Cc | 26.91 | 1 | 0 Aa | 100 | 4 | 0.423±0.250 Bb | 46.64 | 2 | P=0.000, F=32.393, df=17 |
| Chlorpyriphos- ethyl (150 ml $hl^{-1}$) | 0 A* | 100 | 4 | 0 A* | 100 | 4 | 0 A* | 100 | 4 | - |
| Control (Distilled water) | 0.966±0.092 Db | - | | 0.840±046 Da | - | | 0.840±0.046 Ca | - | | P=0.006. F=7.343. df=17 |
| | P=0.000, F=72.458, df=41 | | | P=0.000, F=163.813, df=41 | | | P=0.000, F=19.346, df=41 | | | |

Values followed by the same letter are not statistically different using mean comparisons (Duncan test; $P$<0.05).
Capital letters following the values represent comparisons within a column and lower case letters represent comparisons within a line.
* Insufficient number of insects to evaluate this biological characteristic. [3] Toxicity Class according to (IOBC) classification.

## 4. Conclusion
In conclusion, Chlorpyrifos-ethyl followed by Spinosad were proved to be the most toxic treatments towards all the development stages of *Trichogramma cacoeciae* species. Thus their spraying time must not concur with the most susceptible *T. cacoeciae* development stages. Besides, pesticides in the categories harmless and moderately harmful should be more considered for use in an integrated pest management (IPM) approach.

## 5. Acknowledgments
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