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***Bacillus thuringiensis*, deltamethrin and spinosad side-effects on three *Trichogramma* species**

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Abstract

One biological insecticide, *Bacillus thuringiensis* (Berliner) (Bacillales Bacillacae), and two chemical insecticides, deltamethrin and spinosad, were assessed for their effects on immature stages and adults survival of *Trichogramma cacoeciae* Marchal, *Trichogramma bourarachae* Pintureau et Babault and *Trichogramma evanescens* Westwood (Hymenoptera Trichogrammatidae) from Tunisia. The egg parasitoids were collected on *Ectomyelois ceratoniae* (Zeller) (Lepidoptera Pyralidae) in pomegranate orchard (*Punica granatum* L.). Deltamethrin and spinosad proved to be harmful to the parasitoid development stages and *B. thuringiensis* 100 g hl^{-1} formulation was characterised as slightly harmful only to *T. bourarachae* prepupae stage. However, *B. thuringiensis* 70 g hl^{-1} formulation proved to be harmless towards all the development stages of the tested *Trichogramma* species. As regards insecticides persistence on pomegranate leaves, *B. thuringiensis* actions on all studied *Trichogramma* species were comparable with those of the control, regardless of the day after insecticide treatment. However, spinosad and deltamethrin residues were lethal for all *Trichogramma* adult species, even six days after treatment.

Key words: *Bacillus thuringiensis*, deltamethrin, spinosad, *Trichogramma cacoeciae*, *Trichogramma bourarachae*, *Trichogramma evanescens*, carob moth, pomegranate.

Introduction

In Tunisia, the carob moth *Ectomyelois ceratoniae* (Zeller) (Lepidoptera Pyralidae) is a polyphagous species that infests predominately date palm *Phoenix dactylifera* L. (Arecaceae), pomegranate *Punica granatum* L. (Lythraceae), pistachios *Pistachio vera* L. (Anacardiaceae) and almonds *Prunus amygdalus* L. (Rosaceae). Despite the pollution factor, the chemical control is the most used strategy for pest containment. However, to monitor this species on pomegranate cultivation, insecticides seem to be inefficient and rarely used, due to the endophytic behaviour of the pyralid and the dangling position of the fruit on the pomegranate tree (Dhouibi *et al*., 2000). This led to the adaptation of the strategy of mass-rearing and release of the carob moth natural parasitoids such as *Habrobracon hebetor* (Say) (Hymenoptera Braconidae), *Phanerotoma ocuralis* (Kohl) (Hymenoptera Braconidae) and egg parasitoids of the *Trichogramma* genus (Hymenoptera Trichogrammatidae). The latter are minute wasps that oviposit predominately in Lepidoptera eggs (Losey and Calvin, 1995; Laurent *et al*., 1998; Pintureau *et al*., 1999; 2001; 2003; Stouthamer *et al*., 1999; Rohi and Pintureau, 2003; Pereira *et al*., 2004). They are spread world-wide and play an important role on a broad range of agricultural crops (Hassan and Guo, 1991; Hassan, 1993; 1998; Hafez *et al*., 1999) as biological control agents (Pinto and Stouthamer, 1994; Volkoff *et al*., 1995). They are, in fact, the most widely used insect natural enemies in the world, partly because they are easy to mass rear (Li, 1994; Losey and Calvin, 1995; Ulrichs and Mewis, 2004) and because they kill major crop pests (Hassan, 1998) inside their egg stage before they cause feeding damage (Ulrichs and Mewis, 2004).

In an attempt to record local *Trichogramma* populations, some indigenous species were found on the natural eggs of *E. ceratoniae* and on eggs from the laboratory of the Mediterranean flour moth *Ephestia kuehniella* (Zeller) (Lepidoptera Pyralidae) parasitized eggs in a pomegranate orchard located at the Governorate of Gabes (South-eastern Tunisia; 10°18'E 33°38'N).

In fact, in Tunisian oases, an ancestral method of growing known as “under-level cultivation” continues to be followed. It consists in the cultivation of plants of different heights in the same area. Thus, the first level is represented by date palm trees, the second by pomegranate and grape-vine trees, whereas the third level consists in market gardening. In such complex agrosystems, a balance of nature existed, and in olden times farmers relied only on cultural methods as a means of control. Nonetheless, in recent years and in order to reach the threshold of profitability, pesticides have been used with increasing rate. On the other hand, the use of chemical products that are harmful to natural enemies may drastically reduce or eliminate the naturally occurring entomophagous, causing the outbreak of one or more pests (Cônsoli *et al*., 2001). Indeed, the non-selective use of pesticides leads to the reduction of date palms harvest by up to 20% (Khoualdia *et al*., 1994). Thereby, detailed knowledge of the effects of different pesticides on the immature stages of natural enemies will help to determine the timing of sprays in order to avoid the most susceptible stages (Takada *et al*., 2001). Thus, the side-effects of insecticides have to be studied in order to maximize the compatibility of chemical and biological control methods (Youssef *et al*., 2004).

Therefore, the primary aim of this work was to investigate the side-effects of three biological and chemical insecticides, used with frequency in Tunisian oases, under the ancestral method of growing, on three indigenous *Trichogramma* species. As part of an overall evaluation of local *Trichogramma* species survival in oasis microclimate, studies were conducted on their pre-imaginal development and adult longevity. The tested insecticides, *Bacillus thuringiensis* Berliner (Bacillales Bacillacae) (Formulation: 100 g hl^{-1} and 70 g hl^{-1}), deltamethrin and spinosad were selected on the basis of their utility or potential for lepidopteran control in Tunisian oases.

Materials and methods

The three *Trichogramma* species used in all experiments originated from the parasitized eggs of *E. ceratoniae* or *E. kuehniella* (sentinel egg traps), collected during the summer and the early autumn of 2004 in a pomegranate orchard located in Gabes Governorate. Then, they were identified as *Trichogramma cacoeciae* Marchal, *Trichogramma bourarachae* Pintureau et Babault and *Trichogramma evanescens* Westwood (Ksentini *et al*., 2010). The stock populations of *Trichogramma* species were maintained on the eggs of the factitious host, *E. kuehniella*, under laboratory conditions (25 ± 1°C; 60 ± 10% RH; L/D: 16/8) at "The Olive Tree Institute". *E. kuehniella* moths were produced on diets based on whole wheat flour. The host eggs were UV-killed, glued on cards with diluted Arabic gum and then offered to parasitization. Parasitoids were held under the environmental conditions mentioned above and provided with a (50%) honey solution as a food source throughout the rearing and experimental process.

As regards bioassay on development stages, for all the *Trichogramma* species tested below, tested *E. kuehniella* eggs were glued to a 0.7 cm^2 area (approximately 300 eggs) and exposed to 15 females for 4 hours at (25 ± 1 °C; 60 ± 10% RH; L/D: 16/8). Wasps remaining on eggs at the end of this period were gently discarded with a camelhair brush, then, *E. kuehniella* eggs were transferred to new containers and stored under the conditions mentioned above, until the parasitoids reached the desired developmental stage.

The parasitized eggs obtained were treated by dipping the cards into the insecticidal solutions for 10 seconds either three, six, or nine days after initial parasitism. These days correspond to the larval, prepupal, and pupal stages of *Trichogramma* wasps, respectively (Knutson, 1998). The concentration of each insecticide solution reflected the recommended field rates (table 1), and new solutions were made for each day of exposure. The control group was exposed to distilled water only. After treatment, the cards were kept on filter paper at room temperature until the excess of liquid dried, then transferred to clean vials plugged with cotton, and kept in an incubator (25 ± 1 °C; 60 ± 10% RH; L/D: 16/8). Each treatment was replicated 6 times.

Under the environmental conditions mentioned above, adults generally emerged 10 days after initial parasitism. A final assessment of emergence was made 15 days after initial parasitism in order to check mortality during the immature development. Thus, parasitized eggs (indicated by shiny black appearance of egg chorion) were visually inspected for emergence holes, and those with partially chewed exit holes with dead adults remaining inside were categorized as partially emerged.

To test the persistence of insecticide residues on pomegranate leaves under field conditions, two marked pomegranate branches were sprayed with the equivalent compound at the field-recommended dose (table 1), along with a water control. Sprays were applied with a handgun sprayer in order to make sure that all leaves surfaces were treated. Branches were exposed to direct weather conditions before leaves were excised at intervals from plants. Thus, one, two or six days after applying insecticides, six leaves were detached from each treated and control branch. Quadrate surfaces (1 by 1 cm) were cut out from each leaf and individually placed in vials held at 25 ± 1 °C (60 ± 10% RH; L/D: 16/8) and containing 30 *Trichogramma* adults (<24 hours old) with a drop of (50%) honey solution. Six replicates were used for each treatment. Vials were placed on their sides with the leaf sections placed in their tops and adjacent to a light source to ensure that *Trichogramma* wasps contacted frequently the leaf sections. The number of dead wasps was counted 24 hours after exposure and 10 days after; the total number of individuals was counted to determine the percentage of mortality. This process was repeated on days two and six after treatment. The persistence was the time required for the pesticide residue to lose effectiveness.

All statistical analyses were undertaken with SPSS for Windows Version 13.0 (SPSS, 2004), and all the data were angle transformed and compared by the Tukey's test ($P \leq 0.05$) whenever differences were indicated. Also, mortality occurring during development stages was classified according to the International Organization of Biological Control (IOBC), where : 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%) (Bueno *et al*., 2008).

Table 1. Biological and chemical insecticides, and Recommended Field Rates (RFR) tested on the egg parasitoids *T. cacoeciae*, *T. bourarachae* and *T. evanescens*.

Active ingredient	Trade name	Formulation	RFR
B. thuringiensis (*B.t*)	Bactospeine 16000	WP	100 g hl^{-1}
B. thuringiensis (*B.t*)	Bactospeine 16000	WP	70 g hl^{-1}
Deltamethrin	Decis EC 25	EC	50 cc hl^{-1}
Spinosad	Tracer 240 SC	EC	60 cc hl^{-1}

EC = Emulsifiable Concentrate; WP = Wettable Powder.

Results

Bioassay on development stages

Trichogramma development stages' survival on *E. kuehniella* eggs was significantly affected by chemical treatments. Spinosad and deltamethrin were the most toxic compounds. Even though *B. thuringiensis* 70 g hl^{-1} formulation was found to be harmless to all the development stages of the tested *Trichogramma* species, *B. thuringiensis* 100 g hl^{-1} formulation was characterised as slightly harmful only to *T. bourarachae* prepupae stage (table 2). The results suggested that the susceptibility of the development stages of *Trichogramma* toward insecticides could differ from species to species and the insecticide type used.

Also, we noticed that for almost all tested *Trichogramma* species, parasitized eggs exposed to spinosad displayed the highest mortality rate during adult emergence, followed by eggs exposed to deltamethrin (table 3). However, *T. evanescens* and *T. bourarachae* larvae stages were among the most touched by mortality during adult emergence, whereas for *T. cacoeciae*, this phenomenon was higher for prepuae stages than for larvae and pupae stages, when treated with spinosad. The percentage of partial emergence from eggs exposed to *B. thuringiensis* was similar to that of the control.

Residue bioassay

Adult *Trichogramma* survival was significantly affected by spinosad and deltamethrin treatments. In fact, whatever the day after insecticide treatment; *B. thuringiensis* actions on all studied *Trichogramma* species were comparable with those of the control, whereas deltamethrin and spinosad continued to be harmful to adults during the first, the second and even the sixth day after insecticide pulverisation (table 4).

Discussion

Like other insect species, *Trichogramma* immature stages have long been considered less responsive to insecticides than adults, because the egg chorion is a sort of protection to development stages. However, in this paper, we found that egg chorion protection was not enough to the spinosad and deltamethrin rates tested. In fact, these compounds were not only harmful to all development stages but also to adult *Trichogramma*, as well. In fact, it was found for all tested *Trichogramma* species, that the disruptive effect of deltamethrin and spinosad on emergence was maintained as the parasitoids advanced in development. Our results concur with those obtained by Suh *et al*. (2000), Cônsoli *et al*. (2001) and Bueno *et al*. (2008) who found that immature *Trichogramma* stages exposed to spinosad were characterized by a very high mortality rate.

While the toxicity of some insecticides is subject to controversy, the only possible explanation may be related to the methodology of the study, the host eggs used as well as the insecticide concentrations and/or

Table 2. Mortality (%) from egg to adult of 3 *Trichogramma* species treated with different insecticide solutions during immature life phases (25 ± 1 °C; 60 ± 10% RH; L/D: 16/8).

Trichogramma species / Treatment	Immature developmental stage treated: larvae	class	prepupae	class	pupae	class	
T. bourarachae							
Control	3.74 ± 1.4 Aa	1	7.21 ± 2.42 Ab	1	3.28 ± 0.99 Aa	1	F = 9.417; df = 2; P = 0.002
B.t 100 g hl^{-1}	4.18 ± 1.17 Aa	1	36.53 ± 4.72 Bb	2	3.4 ± 0.75 Aa	1	F = 331.607; df = 2; P = 0.0001
B.t 70 g hl^{-1}	9.29 ± 1.12 Bb	1	4.35 ± 2.32 Aa	1	17.49 ± 3.56 Bc	1	F = 21.569; df = 2; P = 0.0001
Deltamethrin	85.63 ± 3.04 Ca	3	94.52 ± 1.92 Cb	3	95.41 ± 1.25 Cb	3	F = 34.525; df = 2; P = 0.0001
Spinosad	100 ± 0 Da	4	92.98 ± 10.47 Ca	3	100 ± 0 Da	4	F = 3.352; df = 2; P = 0.063
	F = 2693.424; df = 4; P = 0.0001		F = 141.170; df = 4; P = 0.0001		F = 2972.687; df = 4; P = 0.0001		
T. cacoeciae							
Control	2.5 ± 0.41 Aa	1	6.74 ± 0.98 Cb	1	6.61 ± 1.11 Ab	1	F = 60.026; df = 2; P = 0.0001
B.t 100 g hl^{-1}	2.03 ± 0.62 Aa	1	3.07 ± 0.63 Ab	1	8.33 ± 1.04 Ac	1	F = 91.112; df = 2; P = 0.0001
B.t 70 g hl^{-1}	2.84 ± 0.64 Aa	1	4.93 ± 0.56 Bb	1	7.27 ± 0.88 Ac	1	F = 59.384; df = 2; P = 0.0001
Deltamethrin	86.76 ± 2.4 Bc	3	77.52 ± 1.94 Da	2	83.24 ± 1.47 Bb	3	F = 29.182; df = 2; P = 0.0001
Spinosad	94.61 ± 0.75 Cc	3	79.83 ± 2.2 Da	2	87.98 ± 2.17 Cb	3	F = 110.752; df = 2; P = 0.0001
	F = 4110.101; df = 4; P = 0.0001		F = 3078.561; df = 4; P = 0.0001		F = 2794.771; df = 4; P = 0.0001		
T. evanescens							
Control	6.74 ± 1.42 Aa	1	6.91 ± 0.72 Aa	1	9.99 ± 2.23 Ab	1	F = 7.731; df = 2; P = 0.005
B.t 100 g hl^{-1}	6.4 ± 0.48 Aa	1	20.97 ± 1.98 Cc	1	11.67 ± 2.28 Ab	1	F = 110.987; df = 2; P = 0.0001
B.t 70 g hl^{-1}	6.84 ± 1.08 Aa	1	15.66 ± 4.07 Bb	1	14.31 ± 2.3 Ab	1	F = 22.963; df = 2; P = 0.0001
Deltamethrin	90.83 ± 0.71 Bb	3	93.27 ± 2.23 Db	3	69.79 ± 6.55 Ba	2	F = 71.550; df = 2; P = 0.0001
Spinosad	87.16 ± 4.13 Ba	3	93.43 ± 0.81 Db	3	85.74 ± 2.84 Ca	3	F = 13.807; df = 2; P = 0.0001
	F = 1549.587; df = 4; P = 0.0001		F = 1230.016; df = 4; P = 0.0001		F = 476.724; df = 4; P = 0.0001		

Values followed by the same letter are not statistically different using mean comparisons (Tukey test; P < 0.05) angular transformed data, capital letters following the values represent comparisons within a column and lower case letters represent comparisons within a line. Mortality estimation: 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%).

Table 3. Adult mortality during emergence (%) of 3 *Trichogramma* species treated with different insecticide solutions during immature life phases (25 ± 1 °C; 60 ± 10% RH; L/D: 16/8).

Trichogramma species / Treatment	Immature developmental stage treated: larvae	prepupae	pupae	
T. bourarachae				
Control	0 ± 0 A*	0 ± 0 A*	0 ± 0 A*	
B.t 100 g hl^{-1}	0.17 ± 0.42 Aa	0 ± 0 Aa	0.07 ± 0.18 Aa	F = 0.627; df = 2 ;P = 0.547
B.t 70 g hl^{-1}	0.63 ± 0.82 Aa	0.09 ± 0.22 Aa	0 ± 0 Aa	F = 2.985; df = 2 ;P = 0.081
Deltamethrin	14.08 ± 2.01 Bb	1.28 ± 1.02 Aa	0.54 ± 0.67 Aa	F = 173.239; df = 2 ;P = 0.0001
Spinosad	20.43 ± 4.1 Ca	8.06 ± 6.9 Bb	0.34 ± 0.4 Ac	F = 25.869; df = 2 ;P = 0.0001
	F = 235.667; df = 4; P = 0.0001	F = 7.913; df = 4; P = 0.0001	F = 2.737; df = 4; P = 0.051	
T. cacoeciae				
Control	0.04 ± 0.1 Aa	0.03 ± 0.07 Aa	0.11 ± 0.16 Aa	F = 0.668; df = 2 ;P = 0.527
B.t 100 g hl^{-1}	0.1 ± 0.24 Aa	0.1 ± 0.16 Aa	0.41 ± 0.35 ABa	F = 2.897; df = 2 ;P = 0.086
B.t 70 g hl^{-1}	0.08 ± 0.18 Aa	0.3 ± 0.2 ABa	1.12 ± 0.41 Bb	F = 23.273; df = 2 ;P = 0.0001
Deltamethrin	1.54 ± 0.83 Ba	1.68 ± 1.28 Ba	0.87 ± 1.08 ABa	F = 0.973; df = 2 ;P = 0.401
Spinosad	2.49 ± 1.42 Ba	13.47 ± 2.91 Cc	4.99 ± 1.15 Cb	F = 48.654; df = 2 ;P = 0.0001
	F = 13.750; df = 4; P = 0.0001	F = 125.619; df = 4; P = 0.0001	F = 40.243; df = 4; P = 0.0001	
T. evanescens				
Control	0.64 ± 0.71 Aa	0.31 ± 0.41 Aa	0 ± 0 Aa	F = 2.957; df = 2; P = 0.083
B.t 100 g hl^{-1}	0.56 ± 0.56 Aa	0.63 ± 0.57 Aa	0.17 ± 0.4 Aa	F = 1.447; df = 2; P = 0.266
B.t 70 g hl^{-1}	0.18 ± 0.45 Aa	0.91 ± 0.61 Aa	0.85 ± 0.58 Aa	F = 3.425; df = 2; P = 0.060
Deltamethrin	12.32 ± 1.18 Bc	4.67 ± 2.82 Bb	1.07 ± 0.81 Aa	F = 42.182; df = 2; P = 0.0001
Spinosad	20.89 ± 4.26 Cb	9.15 ± 3.65 Ca	11.06 ± 5.89 Ba	F = 8.340; df = 2; P = 0.004
	F = 223.524; df = 4; P = 0.0001	F = 23.435; df = 4; P = 0.0001	F = 24.139; df = 4; P = 0.0001	

Values followed by the same letter are not statistically different using mean comparisons (Tukey test; P < 0.05) angular transformed data, capital letters following the values represent comparisons within a column and lower case letters represent comparisons within a line. * Statistical analysis can not be run (Equal percentages for all data).

Table 4. Mean ± SE percent survival of *Trichogramma* adults 24 h following exposure to insecticides residues on pomegranate leaves at various days after application of field used rates.

Trichogramma species	compounds: Control	*B.t* 100 g hl^{-1}	*B.t* 70 g hl^{-1}	Deltamethrin	Spinosad	
T. bourarachae						
1	80.49 ± 18.88 Ab	93.59 ± 7.89 Ab	84.85 ± 10.22 Ab	9.09 ± 4.14 Ba	15.84 ± 7.46 Aa	F = 92.187; df = 4; P = 0.0001
2	89.3 ± 17.18 Ab	89.6 ± 12.2 Ab	68.56 ± 25.92 Ab	19.25 ± 18.66 Ba	7.24 ± 6.53 Aa	F = 36.915; df = 4; P = 0.0001
6	92.84 ± 14.12 Ac	92.61 ± 2.18 Ac	87.08 ± 4.71 Ac	0 ± 0 Aa	13.35 ± 6.34 Ab	F = 337.322; df = 4; P = 0.0001
	F = 0.799; df = 2;P = 0.468	F = 0.384; df = 2; P = 0.687	F = 2.638; df = 2; P = 0.104	F = 12.815; df = 2; P = 0.001	F = 2.757; df = 2; P = 0.096	
T. cacoeciae						
1	100 ± 0 *c	98.89 ± 2.72 Ac	89.15 ± 18.03 Ac	32.95 ± 13.84 Ba	33.77 ± 32.44 Ab	F = 25.862; df = 4; P = 0.0001
2	100 ± 0 *c	100 ± 0 Ac	100 ± 0 Ac	34.03 ± 22.79 Ba	46.65 ± 11.44 Ab	F = 23.790; df = 4; P = 0.0001
6	100 ± 0 *b	96.69 ± 5.82 Ab	94.72 ± 4.59 Ab	13.47 ± 16.75 Aa	44.16 ± 24.75 Aa	F = 13.847; df = 4; P = 0.0001
		F = 1.956; df = 2; P = 0.176	F = 0.485; df = 2; P = 0.625	F = 4.826; df = 2; P = 0.024	F = 3.281; df = 2; P = 0.066	
T. evanescens						
1	80 ± 44.72 Ac	72.03 ± 20.79 Ac	96.67 ± 7.46 Ac	4.23 ± 6.63 Aa	38.36 ± 44.16 Ab	F = 7.580; df = 4; P = 0.001
2	78.59 ± 19.9 Abc	87.73 ± 17.19 Ac	64.58 ± 50.26 Abc	20.22 ± 23.34 Aab	13.33 ± 18.26 Aa	F = 6.388; df = 4; P = 0.003
6	82.29 ± 13.84 Ab	80.64 ± 11.67 Ab	85.93 ± 13.01 Ab	40.6 ± 39.54 Aa	5.72 ± 9.04 Aa	F = 15.066; df = 4; P = 0.0001
	F = 0.311; df = 2; P = 0.732	F = 1.845; df = 2; P = 0.204	F = 0.659; df = 2; P = 0.536	F = 1.435; df = 2; P = 0.279	F = 1.213; df = 2; P = 0.334	

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formulations tested in each of the studies. In fact, Grützmacher *et al*. (2004) demonstrated that the sensitivity of *Trichogramma* immature stages was due to the insecticide used. For example, Assist® was found to be harmless to all parasitoid development stages, Dipterex® 500 was slightly harmful to the parasitoid eggs and moderately harmful to the other two stages, whereas Lebaycid® 500 was harmful to the parasitoid egg and larvae and moderately harmful to the pupae within the host eggs. However, in our case, we observed that the insecticide action might be strongly related to the species of *Trichogramma* used in the test. Indeed, *B. thuringiensis* 100 g hl^{-1} formulation was found to be slightly harmful only to *T. bourarachae* prepuae stages.

As regards the insecticide effects on the adults emerging from the host eggs, we found that while treated with spinosad, *T. evanescens* and *T. bourarachae* larvae stages were among the most susceptible, whereas for *T. cacoeciae*, this phenomenon was higher for prepuae stages than larvae and pupae stages. This might depend on *Trichogramma* species. For example, Suh *et al*. (2000) found when testing spinosad effects on *Trichogramma exiguum* Pinto et Platner premature stages that the partial emergence level increased while preimaginal development progressed. In an attempt to explain the partial emergence phenomenon, Cônsoli *et al*. (2001) reported that some products might be unable to penetrate through the host chorion. However, their ingestion by the parasitoid might occur during the opening of the emergence hole. In fact, while the parasitoid is cutting a small area of the host chorion with its mandibles, a small quantity of the chorion surface could be swallowed and with it the product covering the host surface. He also demonstrated that the characteristic mortality of the newly emerged adults could be explained by the mode of action of spinosad, which excites the nervous system by activating the nicotinic receptors of acetylcholine, thereby causing paralysis. In our study, as for spinosad, the majority of parasitized eggs exposed to deltamethrin did not produce viable adults. In fact, newly emerged *Trichogramma* adults died while chewing exit holes through the egg chorion. This might be due to the deltamethrin's contact and/or stomach-acting modes.

Concerning the insecticide effects on *Trichogramma* adult survival, deltamethrin and spinosad residues were found to be very toxic towards all *Trichogramma* species tested, whereas *B. thuringiensis* (Formulation: 100 g hl^{-1} and 70 g hl^{-1}) effect was comparable to that of the control. Regarding spinosad, its toxicity towards natural enemies is subject to controversy. In fact, it is considered as promising for IPM programmes because of its selectivity against different beneficial insects such as predatory mites, predatory heteroptera and Neuroptera (Medina *et al*., 2003) or as an insecticide that exhibits wide margins of safety to beneficial insects and related organisms (Babul Hossain and Poehling, 2006). However, these authors found that spinosad showed highly detrimental effects on the development and longevity of the parasitoid *Neochrysocharis formosa* (Westwood) (Hymenoptera Eulophidae) even when the host larvae *Liriomyza sativa* Blanchard (Diptera Agromyzidae) stopped feeding following parasitization. In the same context, Williams *et al*. (2003) reported that Hymenopteran parasitoids are significantly more susceptible to spinosad than predatory insects. They also added that predators generally suffer insignificant sub-lethal effects following exposure to spinosad, whereas parasitoids often show sub-lethal effects including loss of reproductive capacity and reduced longevity. In this way, it became obvious that spinosad was highly toxic to parasitoid wasps. This was also the case of deltamethrin which appeared in our study as very toxic towards *T. bourarachae*, *T. evanescens* and *T. cacoeciae*. Our findings also concur perfectly with those of Youssef *et al*. (2004) who proved that this pyrethroid was very toxic to the adult and pupae of *T. cacoeciae*. Concerning the biological insecticide *B. thuringiensis*, the 100 g hl^{-1} formulation was queerly found in our study to be slightly harmful towards *T. bourarachae* prepupae stage. Also, Brunner *et al*. (2001) found that this compound was responsible for *Trichogramma platneri* Nagarkatti mortality. However, this seemed to be rather due to the physical properties of *B. thuringiensis* formulations which caused the adhesion of wings, legs and antennae to the body, thereby disabling the parasitoids and causing their death.

In this study, the impact of deltamethrin and spinosad was not significantly affected by the time devoted to the experiment after insecticide application. In fact, even six days after pomegranate leaves spraying, these two compounds had a lethal effect on adult *Trichogramma*. Our data confirm the high susceptibility of *Trichogramma* wasps to deltametrin and spinosad and demonstrate that *B. thuringiensis* is more compatible and/or more suitable to conserve natural or released populations of *Trichogramma* wasps. Moreover, beneficial organisms that occupy pomegranate canopy, as well as those which live on or beneath the soil, will be reached by pesticide fatal effect. For example, when spraying deltamethrin on maize leaves, some soil arthropods such as Nitidulidae were significantly affected and even suppressed (Badji *et al*., 2007). Insect suppression by deltamethrin's broad-spectrum indicates their high susceptibility to this insecticide. This was also the case of the tested *Trichogramma* species which continued to be mortally affected by deltamethrin and spinosad even six days after their application. However, according to Suh *et al*. (2000), *T. exiguum* adults are susceptible to residues of spinosad weathered on cotton leaves only up to four days. In this paper, spinosad and deltamethrin long-term persistence might be due to trial conditions, because the sprayed pomegranate leaves were chosen in a permanently shadowed area of the tree.

In our study, *Trichogramma* immature stages and adults received the recommended field dose of insecticides. *B. thuringiensis* was proven to be slightly harmful towards *T. Bourarachae* prepuae stage, although Brunner *et al*. (2001) observed that these kinds of products could be successfully integrated into biological control programmes in conjunction with small parasitoids. Nevertheless, it is useful to bear in mind that under field conditions, eggs would receive a much lower dose, and many eggs such as those oviposited on the underside of leaves might not even be exposed to the insecticide (Suh *et al*., 2000).

Conclusion

Further research should focus on the impact of insecticide exposure on native *Trichogramma* species present in traditional oases in Gabes area. Pesticides that control pests without adversely affecting important natural enemies are urgently needed for integrated control programmes (Hafez *et al*., 1999). Without such insecticides, necessity will certainly continue to force some farmers to apply for conventional agriculture with all its arsenal of harmful products. In such cases, the use of insecticides with short-lived residues would theoretically allow a rapid recolonization of parasitoids (Brunner *et al*., 2001). Nevertheless, the use of effective and harmless biological compounds will be, in the long run, safer and more useful, for farmers, consumers, ecosystem and of course beneficial organisms.

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References

BABUL HOSSAIN M., POEHLING H. M., 2006.- Non-target effects of three biorationale insecticides on two endolarval parasitoids of *Liriomyza sativa* (Dip., Agromyzidae).- *Journal of Applied Entomology*, 130: 360-367.

BADJI C. A., GUEDES R. N. C., SILVA A. A., CORRÊA A. S., QUEIROZ M. E. L. R., MICHEREFF-FILHO M., 2007.- Non-target impact of deltamethrin on soil arthropods of maize fields under conventional and no-tillage cultivation.- *Journal of Applied Entomology*, 131: 50-58.

BRUNNER J. F., DUNLEY J. E., DOERR M. D., BEERS E. H., 2001.- Effect of pesticides on *Colpoclypeus florus* (Hymenoptera: Eulophidae) and *Trichogramma platneri* (Hymenoptera: Trichogrammatidae), parasitoids of leafrollers in Washington.- *Journal of Economic Entomology*, 94: 1075-1084.

BUENO A. F., BUENO R. C. O. F., PARRA J. R. P., VIEIRA S. S, 2008.- Effects of pesticides used in soybean crops to the egg parasitoid *Trichogramma pretiosum*.- *Ciência Rural*, 38: 1495-1503.

CÔNSOLI F. L., BOTELHO P. S. M., PARRA J. R. P., 2001.- Selectivity of insecticides to the egg parasitoid *Trichogramma galloi* Zucchi, (1988) (Hym., Trichogrammatidae).- *Journal of Applied Entomology*, 125: 37-43.

DHOUIBI M. H., HAWLITSKY N., ZAARAOUI H., KRISAANE T., CHEIKH T., CHERNI M., BEN MOUSSA I., 2000.- Biological control against the carob moth *Ectomyelois ceratoniae* in oases and in packing houses in Tunisia, pp. 229-236. In: *Area-wide control of fruit flies and other insect pests* (TAN K. H., Eds).- Penerbit Universiti Sains Malaysia, Pulau Penang, Malaysia.

GRÛTZMACHER A. D., ZIMMERMANN O., YOUSSEF A., HASSAN S. A., 2004.- The side-effects of pesticides used in integrated production of peaches in Brazil on the egg parasitoid *Trichogramma cacoeciae* Marchal (Hym., Trichogrammatidae).- *Journal of Applied Entomology*, 128: 377-383.

HAFEZ M. B., SCHMITT A., HASSAN S. A., 1999.- The side effects of plant extracts and metabolites of *Reynoutria sachalinensis* (F. Schmidt) Nakai and conventional fungicides on the beneficial organism *Trichogramma cacoeciae* Marchal (Hym., Trichogrammatidae).- *Journal of Applied Entomology*, 123: 363-368.

HASSAN S. A., 1993.- The mass rearing and utilization of *Trichogramma* to control Lepidopterous pests: Achievements and outlook.- *Pesticide Science*, 37: 387-391.

HASSAN S. A., 1998.- Standard laboratory methods to test the side-effects of pesticides (initial and persistent) on *Trichogramma cacoeciae* Marchal (Hym., Trichogrammatidae), pp. 71-79. In: *Ecotoxicology: pesticides and beneficial organisms* (HASKELL P. T., MCEWEN P., Eds).- Chapman & Hall, London, UK.

HASSAN S. A., GUO M. F., 1991.- Selection of effective strains of egg parasites of the genus *Trichogramma* (Hym., Trichogrammatidae) to control the European corn borer *Ostrinia nubilalis* Hb. (Lep., Pyralidae).- *Journal of Applied Entomology*, 111: 335-341.

KHOUALDIA O., MARRO J. P., BRUN J., 1994.- Release essays of *Phanerotoma ocuralis* Kohll (Hym., Braconidae) against the carob moth *Ectomyelois ceratoniae* Zeller (Lep., Pyralidae) in Tozeur, pp. 67-69. In: *Premier séminaire Franco-Tunisien sur l'Agronomie oasienne* (INSTITUT DES RÉGIONS ARIDES DE MEDENINE, Eds).- Centre de Recherches Phoénicicoles de Degache, Tunisie (In French).

KNUTSON A., 1998.- *The* Trichogramma *manual*.- Texas Agriculture Extension Service, Texas A&M University System, College Station, Texas, USA.

KSENTINI I., MONJE J. C., 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.

LAURENT V., WAJNBERG E., MANGIN B., SCHIEX T., GASPIN C., VANLERBERGHE-MASUTTI F., 1998.- A composite genetic map of the parasitoid wasp *Trichogramma brassicae* based on RAPD markers.- *Genetics*, 150: 275-282.

LI L. Y., 1994.- Worldwide use of *Trichogramma* for biological control on different crops: a survey, pp. 37-51. In: *Biological control with egg parasitoids* (WAJNBERG E., HASSAN S. A., Eds).- Cab international, Oxon, UK.

LOSEY J. E., CALVIN D. D., 1995.- Quality assessment of four commercially available species of *Trichogramma* (Hymenoptera: Trichogrammatidae).- *Journal of Economic Entomology*, 88: 1243-1250.

MEDINA P., BUDIA F., DEL ESTAL P., VINUELA E., 2003.- Effects of three modern insecticides, pyriproxyfen, spinosad and tebufenozide, on survival and reproduction of *Chrysoperla carnea* adults.- *Annals of Applied Biology*, 142: 55-61.

PEREIRA J. A., BENTO A., CABANAS J. E., TORRES L. M., HERZ A., HASSAN S. A., 2004.- Ants as predators of the egg parasitoid *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae) applied for biological control of the olive moth, *Prays oleae* (Lepidoptera: Hyponomeutidae) in Portugal.- *Biocontrol Science and Technology*, 14: 653-664.

PINTO J. D., STOUTHAMER R., 1994.- Systematics of the Trichogrammatidae with emphasis on *Trichogramma*, pp. 1-35. In: *Biological control with egg parasitoids* (WAJNBERG E., HASSAN S. A., Eds).- CAB International, Oxon, UK.

PINTUREAU B., CHAPELLE L., DELOBEL B., 1999.- Effects of repeated thermic and antibiotic treatments on a *Trichogramma* (Hym., Trichogrammatidae) symbiont.- *Journal of Applied Entomology*, 123: 473-483.

PINTUREAU B., LASSABLIÈRE F., DAUMAL J., GRENIER S., 2001.- Does a cyclic natural thermal cure occur in *Wolbachia*-infected *Trichogramma* species?- *Ecological Entomology*, 27: 366-372.

PINTUREAU B., PIZZOL J., BOLLAND P., 2003.- Effects of endosymbiotic *Wolbachia* on the diapause in *Trichogramma* hosts and effects of the diapause on *Wolbachia*.- *Entomologia Experimentalis et Applicata*, 106: 193-200.

ROHI L., PINTUREAU B., 2003.- Are *Trichogramma bourarachae* and the *perkinsi* species group really distinct from *Trichogramma buesi* and the *pintoi* group, respectively?- *Journal of Applied Entomology*, 127: 265-268.

SPSS, 2004.- *SPSS 13.0 brief guide*.- SPSS Incorporation, Chicago, USA.

STOUTHAMER R., HU J., VAN KAN F. J. P. M., PLATNER G. R., PINTO J. D., 1999.- The utility of internally transcribed spacer 2 DNA sequences of the nuclear ribosomal gene for distinguishing sibling species of *Trichogramma*.- *Biocontrol*, 43: 421-440.

SUH C. P. C., ORR D. B., VAN DUYN J. W., 2000.- Effect of insecticides on *Trichogramma exiguum* (Trichogrammatidae: Hymenoptera) preimaginal development and adult survival.- *Journal of Economic Entomology*, 93: 577-583.

TAKADA Y., KAWAMURA S., TANAKA T., 2001.- Effects of various insecticides on the development of the egg parasitoid *Trichogramma dendrolimi* (Hymenoptera: Trichogrammatidae).- *Journal of Economic Entomology*, 94: 1340-1343.

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).- *Journal of Applied Entomology*, 128: 426-431.

VOLKOFF A. N., DAUMAL J., BARRY P., FRANÇOIS M. C., HAWLITZKY N., ROSSI M. M., 1995.- Development of *Trichogramma cacoeciae* Marchal (Hymenoptera: Trichogrammatidae): time table and evidence for a single larval instar.- *International Journal of Insect Morphology and Embryology*, 24: 459-466.

WILLIAMS T., VALLE J., VINUELA E., 2003.- Is the naturally derived insecticide spinosad® compatible with insect natural enemies?- *Biocontrol Science and Technology*, 13: 459-475.

YOUSSEF A. I., NASR F. N., STEFANOS S. S., ELKHAIR S. S. A., SHEHATA W. A., AGAMY E., HERZ A., HASSAN S. A., 2004.- The side-effects of plant protection products used in olive cultivation on the hymenopterous egg parasitoid *Trichogramma cacoeciae* Marchal.- *Journal of Applied Entomology*, 128: 593-599.

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