

Effect of Insecticides on *Trichogramma* Parasitoids Used in Biological Control against *Prays oleae* Insect Pest

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Abstract

This study investigated the direct effect of the insecticides deltamethrin and spinosad on three egg parasitoids species: *Trichogramma oleae*, *T. cacoeciae* and *T. bourarachae*. The parasitoid pupae were exposed to pesticide residues on fresh olive tree leaves at recommended concentrations (RC) at different time intervals: 3, 10, 17, 24 and 31 days after pesticide applications. Parasitism viability (% emergence from parasitized eggs) and adult emergence time (developmental time from pupa to adult emergence) were evaluated. Regarding to the International Organization of Biological Control (IOBC) guidelines, results of toxicity effects of insecticides show that: Deltamethrin was moderately harmful to all *Trichogramma* species at RC (Decis[®] 100 milliliters·ha⁻¹), however, spinosad was harmless to moderately harmful at RC (Tracer[®] 20 milliliters·ha⁻¹). *Trichogramma* species revealed differences with regard to adult emergence time and exhibited significant changes in parasitism viability with increasing time after pesticide treatment. While deltamethrin residues affected parasitism viability 31 days after the product application, spinosad displayed similar viability for almost species 24 days after the application. The usefulness of *Trichogramma* parasitoids used as biological control agents, in olive tree ecosystem, was discussed in integrated pest management programs for *Prays oleae* control when parasitoid species were exposed during pupal stage to the insecticide residue.

Keywords

Insecticide Effect, *Trichogramma* Species, Olive Tree, Parasitism Viability, Emergence Time

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1. Introduction

Integrated Pest Management (IPM) is an effective and environmentally approach to pest management that relies on a combination of chemical and biological control methods. Of particular interest, parasitoids of the genus *Trichogramma*, which are egg-parasitoids in the family Trichogrammatidae, have been reported to be efficient in regulating populations of lepidopteran pests in agricultural cropping systems [1] [2]. Among main lepidopteran insect pest of olive tree, the olive moth *Prays oleae* Bern (Lepidoptera, Hyponomeutidae) is the most abundant throughout the Mediterranean and the Black Sea, the Middle East and the Canary Islands [3]. In Tunisia, the damage caused by *P. oleae* can reduce olive production by 50% [4]. As a consequence, efforts to develop biological control methods in IPM programs were initiated through the release of egg parasitoids and use of pheromones. However, release and integration issues that are considered important for the development of a successful IPM include important factors such as the pesticide choice and the timing of application [5]. In fact, *Trichogramma* is generally sensitive to pesticides [6] and most chemical products can be immediately toxic (contact toxicity) or it can persist (toxicity of dried residue) [7] [8]. Moreover, contact with pesticides at the less susceptible life stage (parasitoids within hosts) can cause prolonged development time of immature stages and reduced emergence from parasitized eggs [9]. Accordingly, this is important to determine the compatibility of parasitoids with pesticides for IPM programs.

Saccharopolyspora spinosa [10] or spinosad is a product classified as an environmentally and toxicologically reduced risk material [11]. Spinosad-based products have been registered in more than 30 countries for control of pest: Lepidoptera, Diptera, some Coleoptera, ants and thrips [12]. As a biorational pesticide, spinosad now represents an important option for pest control in a growing number crops produced under systems of integrated pest management (IPM) [13]. A recent review of predator and parasitoid susceptibility to spinosad concluded that this product represented one of the most judicious insecticides available for the conservation of predator populations [14]. However, the majority of laboratory and field studies report moderately harmful or harmful effects on populations of hymenopteran parasitoids [15]-[17]. Spinosad is commercially available under the name of Tracer 240[®] and is used against the first and second generations of *P. oleae* in most olive groves in Tunisia. However larval control of the first and the second generation of *P. oleae* usually rely on insecticides when the damage threshold reaches 5% for flowers and 20% for fruits. Pesticide persistence and mobility are influenced by the properties of the pesticide as well as by the soil environment, site conditions, weather and application methods. Therefore, information on degradation rate also helps to assess and predict the environmental behaviour of the pesticide.

Primary insecticide used is the pyrethroid, deltamethrin (Decis[®]), which is valued for its residual activity, persistence and high toxicity to terrestrial invertebrates [18]. Generally, the second generation is difficult to manage with insecticides because larvae bore into the fruit immediately after emerging from the eggs [19]. Other control measures are than needed and a potential alternative strategy is the release of *Trichogramma* species. In fact, this is a way to inhibit first larval stage of *P. oleae* on fruits [5]. However, one factor that may inhibit the ability of *Trichogramma* species to regulate pest population is the toxic insecticide residues.

Today, more than studying the toxic effect of pesticides on pests, there is a trend to evaluate their innocuousness towards natural enemies; These residues may be found in the soil, groundwater or the sediment of waterways and may also be found in vertebrates/invertebrates associated with the treated area, through direct contact or food chain effects [20].

The selection of the most adequate parasitoid species is vital for any program of biological control [21]. It is important to select the appropriate *Trichogramma* species that are most adapted to the local field conditions for biological control programs. In fact, according to [22], native *Trichogramma* species, which are generally adapted to environment, are better biological control agents. Moreover, quality measures that can be associated with the effectiveness of *Trichogramma* species to control the target pest are attributed mainly to the parasitism viability and to the developmental time from egg to adult emergence [23]. Therefore, the present study was designed to determine the side-effects of two insecticides deltamethrin (Decis[®]) and spinosad (Tracer 240[®]) on three native *Trichogramma* species *T. oleae*, *T. cacoeciae* and *T. bourarachae* [24] and to discuss their possible use in control programs against the second generation of *P. oleae* in olive grove areas where biological control has limited use.

2. Materials and Methods

2.1. Rearing of *Trichogramma* Parasitoid

Trichogramma species: *T. oleae*, *T. cacoeciae* and *T. bourarachae* used for the experiment were reared under

laboratory conditions on *Ephesia kuehniella* eggs. Glued cardboard squares (1 cm²), each with approximately 250 *E. kuehniella* eggs, were presented for 24 hours to recently emerged *Trichogramma* females (20 females per card) in glass tubes (6 cm × 1.5 cm) at 20°C, 60% RH and 16L: 8D. The cards with a parasitism rate close to 100% were then transferred to other glass tubes and maintained until pupation (192 hours).

2.2. Treatment of Olive Trees with Insecticide

Two years young olive trees maintained in greenhouse at 25°C, 70% RH were used for the sampling. Five olive trees per insecticide were sprayed till run-off with a hand sprayer at the same day (3 litres of solution per tree) and an eleventh tree sprayed with water for the control. Treatments were applied according to the recommended label rate for control of *P. oleae*. The insecticides tested were: deltamethrin at 3.75 grams·ha⁻¹ (Decis® 100 milliliters·ha⁻¹) and spinosad at 24 grams·ha⁻¹ (Tracer® 20 milliliters·ha⁻¹).

Olive leaves were collected at 3, 10, 17, 24 and 31 after olive trees spraying and used for exposure tests of parasitoid pupae at intervals after treatment.

2.3. Exposure Tests of Parasitoid Pupae at Intervals after Treatment

In each time after the treatment (at 3, 10, 17, 24 and 3 days), a card with pupae parasitoid was placed inside transparent plastic petri dish (6 cm × 4 cm) with treated (using the insecticide) or with control (using water) olive leaf samples. A 2 × 2 cm aeration hole (4 cm²) covered with fine cotton cloth was made on the petri dish to assure continued ventilation in the area. In total, 30 cards for each *Trichogramma* specie (Each card represented a single independent replicate): 15 cards (n = 15 per treatment) and 15 cards (n = 15 per control) were conducted at 25°C ± 2°C, 60% ± 5% RH, and 14 L: 10 D.

2.4. Analyses of Pesticide Residues

2.4.1. Olive Leaf Sampling

Other samples of olive leaves were collected from same young olive trees maintained in greenhouse at each time after the treatment: starting from the day of spray (one hour after pesticide application) and at different time intervals (3, 10, 17, 24 and 31 days). Leaf samples were collected randomly from each tree and cut in fine pieces (about 5 mm diameter), placed immediately in a cool-box held at 4°C - 8°C after collection and transferred to refrigerator for analysis within 24 - 36 hours.

2.4.2. Extraction

Fifty gram samples collected at each time intervals were blended in 100 ml of acetonitrile–water (80/20; v/v) for 2 min, shaken for approximately 30 min and filtered through a buchner funnel than washed with acetonitrile–water mixture (8:2, v/v). Extract was purified using silica SPE (Solid Phase Extraction) cartridge. The acetonitrile solution was eluted, collected and immediately evaporated to dryness in a rotary vacuum evaporator. The residue was reconstituted in 1 mL of methanol: acetonitrile: 2% ammonium acetate solution (1:1:1, v/v) for final HPLC analysis (HPLC, a Beckman model 322) equipped with UV detector (250 nm). The column used was C18 (250 mm × 4.6 mm i.d). The mobile phase was acetonitrile: methanol: 2% ammonium acetate (21:21:8) at a flow rate of 2.0 mL·min⁻¹. A 5 µL aliquot of each sample was injected each time for residue analysis. The representative retention times of spinosyn A and D were 9.5 min and 11.0 min, respectively.

2.5. Data Analyses

Parasitism viability (number of emerged adults from parasitized pupae) and adult emergence time (developmental time from pupa to adult emergence) were evaluated.

The effect of the insecticides was established by comparing the controls with the treatments using the following formula:

$$E(\%) = (1 - Vt / Vc) \times 100$$

where *E* is the insecticide effect in parasitism viability (number of emerged adults from parasitized pupae) compared to the control; *Vt* is parasitism viability observed for each insecticide treatment and *Vc* is the parasitism viability observed in the control ones. The value *E* calculated for the insecticide treatments were classified ac-

cording 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 ($E > 99\%$) [25]. Proportional data were normalized through arcsine transformation; all the other data were normalized through square root transformation.

The tests were performed using the Statistical Package for the Social Sciences (SPSS) for Windows (version 17.0). A complete randomized factorial design was used, and data were submitted to analysis of variance (ANOVA, $p < 0.05$). Significant mean values associated with parasitism viability were compared by Dunnett's test.

Tukey test for multiple comparisons was performed on the various groups to determine whether differences associated with adult emergence time existed between treatments and exposure periods.

In the text and figures, mean \pm SD (standard deviation) are used as descriptive statistics for distributed data

3. Results

3.1. Insecticide Effect on *Trichogramma* Species

Results from the experiment are presented in **Table 1**. The dominant class was 2 ($30\% < E < 79\%$) for all treatments and parasitoid species combinations. However, the classification changed from class 2 to class 1 ($E < 30\%$) or to class 3 ($80\% < E < 99\%$). In fact, result from treatment using olive leaf samples on day 31 after deltamethrin spraying has show a slightly harmful effect on parasitism viability, except the case for *T. oleae* where the insecticide was harmless ($E\% = 23.6 \pm 8.3$). However, deltamethrin was moderately harmful to *T. bourarachae* ($E\% = 80.5 \pm 2.2$) on day three. Spinosad was harmless to *T. oleae* and *T. cacoeciae* at 10 days ($E\% = 18.8 \pm 9.0$). However, it was slightly harmful to *T. bourarachae* even on day 31 ($E\% = 32.3 \pm 4.8$).

3.2. Comparison between Insecticide Effects on *Trichogramma* Species Parasitism Viability

Table 2 presents the pair multiple comparisons among the treatments associated with parasitism viability for the different assessment periods.

The spinosad treatment significantly reduced parasitism viability of all three *Trichogramma* species for all assessment periods. Difference in the mean values associated with parasitism viability between spinosad treatment and control were 30.7 for *T. bourarachae*, 22.0 for *T. oleae* and 26.0 for *T. cacoeciae* ($P < 0.05$). In addition, parasitism viability of all the three *Trichogramma* species was less affected by spinosad than by deltamethrin ($P < 0.05$).

3.3. Adult Emergence Time of *Trichogramma* Species at the Different Exposure Periods

Adult emergence time (Pupa-adult development time) of *Trichogramma* reared on *E. kuehniella* eggs differed among the three species in their response to insecticide residues at the different exposure periods. Periods of the experiment in the control group do not affect adult emergence time of: *T. oleae* (**Table 3**, Tukey test, $F = 0.62$; $df = 4$; $p = 0.854$), *T. bourarachae* ($F = 0.5$; $df = 4$; $p = 0.736$) and *T. cacoeciae* ($F = 0.71$; $df = 4$; $p = 0.780$). Wasp development from pupa to adult emergence at 25°C normally requires about 6 days. However, pupa exposed to the insecticide residues required about one to two days longer to complete development. Adult emergence time of *T. oleae* was significantly long when pupa were exposed to deltamethrin residues at 3 days after the treatment. More significant decrease in adult emergence time was observed at 10 and 17 days then at 24 and 31 days (**Table 3**, Tukey test, $F = 91, 45$; $df = 4$; $p < 0.05$). However, adult emergence time from *T. oleae* pupa exposed to spinosad residues was significantly decreased at 24 days after the treatment (**Table 3**, Tukey test, $F = 31.67$; $df = 4$; $p < 0.05$).

Adult emergence time from *T. cacoeciae* pupa decreased when exposure period to deltamethrin and to spinosad residues occurred, respectively, at 31 days (**Table 3**, Tukey test, $F=42.27$; $df = 4$; $p < 0.05$) and at 10 days (**Table 3**, Tukey test, $F = 29.83$; $df = 4$; $p < 0.05$) after the treatment.

In contrast, adult emergence time from *T. bourarachae* pupa exposed to deltamethrin or to spinosad residues was similar in all the periods of exposure (**Table 3**, Tukey test, $F = 0.72$; $df = 4$; $p = 0.624$ and $F = 0.52$; $df = 4$; $p = 0.736$).

Table 1. Insecticide effect (E) on parasitism viability reduction associated with *T. oleae*, *T. cacoeciae* and *T. bourarachae* after exposure of parasitoid pupae to residues on leaves at different times (days).

Species	Treatment (grams·ha ⁻¹)	Exposure time	E ¹ (%)	Class ²
<i>T. oleae</i>	Deltamethrin	3 days	62.1 ± 6.2	2
		10 days	66.7 ± 8.1	2
		17 days	49.6 ± 0.2	2
		24 days	41.0 ± 5.8	2
		31 days	23.6 ± 8.3	1
	Spinosad	3 days	52.9 ± 1.8	2
		10 days	18.8 ± 9.0	1
		17 days	16.1 ± 2.6	1
		24 days	14.4 ± 2.9	1
		31 days	10.8 ± 4.7	1
<i>T. cacoeciae</i>	Deltamethrin	3 days	65.6 ± 13.3	2
		10 days	56.9 ± 4.8	2
		17 days	54.4 ± 8.4	2
		24 days	51.0 ± 5.5	2
		31 days	51.6 ± 3.2	2
	Spinosad	3 days	53.1 ± 12.3	2
		10 days	47.5 ± 7.2	2
		17 days	39.2 ± 16.6	2
		24 days	23.6 ± 16	1
		31 days	16.5 ± 12.8	1
<i>T. bourarachae</i>	Deltamethrin	3 days	80.5 ± 2.2	3
		10 days	65.1 ± 6.0	2
		17 days	48.6 ± 9.4	2
		24 days	46.2 ± 7.6	2
		31 days	44.2 ± 3.2	2
	Spinosad	3 days	71.0 ± 2.0	2
		10 days	45.6 ± 11.6	2
		17 days	42.8 ± 13.5	2
		24 days	33.5 ± 14.3	2
		31 days	32.3 ± 4.8	2

¹E(%) = (1 - Vt/Vc) × 100, where E is the effect of the insecticide on parasitism viability reduction compared to the control, Vt is the parasitism viability for the treatments (using insecticide) and Vc is the parasitism viability for the control (using water). ²Class: 1. harmless ($E < 30\%$); 2. slightly harmful ($30\% < E < 79\%$); 3. moderately harmful ($80\% < E < 99\%$); 4. harmful ($E > 99\%$).

Table 2. Comparison between the deltamethrin and spinosad treatments based on parasitism viability of *Trichogramma* species.

Species	Treatment	Parasitism viability Mvd* (control-treatment)	Signification
<i>T. oleae</i>	Spinosad	22.071*	0.000
<i>T. cacoeciae</i>	Spinosad	26.017*	0.000
<i>T. bourarachae</i>	Spinosad	30.712*	0.000
<i>T. oleae</i>	Deltamethrin	34.901*	0.000
<i>T. cacoeciae</i>	Deltamethrin	36.007*	0.000
<i>T. bourarachae</i>	Deltamethrin	35.002*	0.000
(Deltamethrin-spinosad)			
<i>T. oleae</i>		17.212*	0.000
<i>T. cacoeciae</i>		19.314*	0.000
<i>T. bourarachae</i>		23.059*	0.000

Mvd. Mean value differences *Significant difference ($p < 0.05$, Dunnett's test).

Table 3. Effect of deltamethrin and spinosad treatments on the pupa-adult development time (adult emergence time) of *Trichogramma* species after the different exposure periods.

<i>Trich. species period</i>	Exposure	Adult emergence time (days) Deltamethrin	(\pm SD) ^a Spinosad	Control
<i>T. oleae</i>	3 days	8.10 \pm 0.14 ^a	7.07 \pm 0.23 ^a	6.05 \pm 0.19 ^a
	10 days	7.80 \pm 0.45 ^b	6.93 \pm 0.31 ^a	6.22 \pm 0.22 ^a
	17 days	7.20 \pm 0.52 ^b	6.86 \pm 0.51 ^a	6.35 \pm 0.51 ^a
	24 days	6.00 \pm 0.41 ^c	6.03 \pm 0.35 ^b	5.80 \pm 0.47 ^a
	31 days	6.41 \pm 0.33 ^c	6.16 \pm 0.51 ^b	6.25 \pm 0.45 ^a
<i>T. cacoeciae</i>	3 days	7.73 \pm 0.59 ^a	7.71 \pm 0.50 ^a	6.56 \pm 0.51 ^a
	10 days	7.53 \pm 0.51 ^a	7.17 \pm 0.33 ^b	6.49 \pm 0.11 ^a
	17 days	7.50 \pm 0.13 ^a	6.86 \pm 0.81 ^b	6.33 \pm 0.50 ^a
	24 days	6.61 \pm 0.74 ^b	6.29 \pm 0.21 ^c	6.37 \pm 0.54 ^a
	31 days	06.09 \pm 0.45 ^b	6.00 \pm 0.53 ^c	6.13 \pm 0.63 ^a
<i>T. bourarachae</i>	3 days	7.30 \pm 0.12 ^a	7.06 \pm 0.70 ^a	6.13 \pm 0.74 ^a
	10 days	7.28 \pm 0.20 ^a	7.09 \pm 0.79 ^a	6.03 \pm 0.96 ^a
	17 days	7.51 \pm 0.02 ^a	6.86 \pm 0.90 ^a	6.00 \pm 0.94 ^a
	24 days	7.57 \pm 0.80 ^a	6.80 \pm 0.45 ^a	6.06 \pm 0.74 ^a
	31 days	07.21 \pm 0.17 ^a	6.76 \pm 0.79 ^a	6.00 \pm 0.53 ^a

a. Numbers in column followed by different letters are significantly different (Tukey test: $p < 0.05$).

3.4. Parasitism Viability of *Trichogramma* Species Based on the Treatment Periods

Percentage of reduction in parasitism viability of the three *Trichogramma* species differed between treatment and exposure time (days after spraying leaves). **Figures 1-3** present percentages of parasitism viability reduction for each *Trichogramma* specie and corresponding exposure times. It appears that exposure time after insecticide spray had a significant effect on *Trichogramma* parasitism viability.

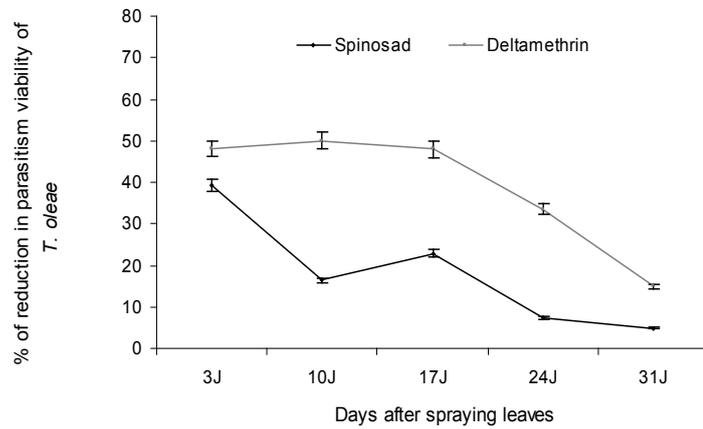


Figure 1. Reduction in parasitism viability (%) of *T. oleae* at days after spraying leaves.

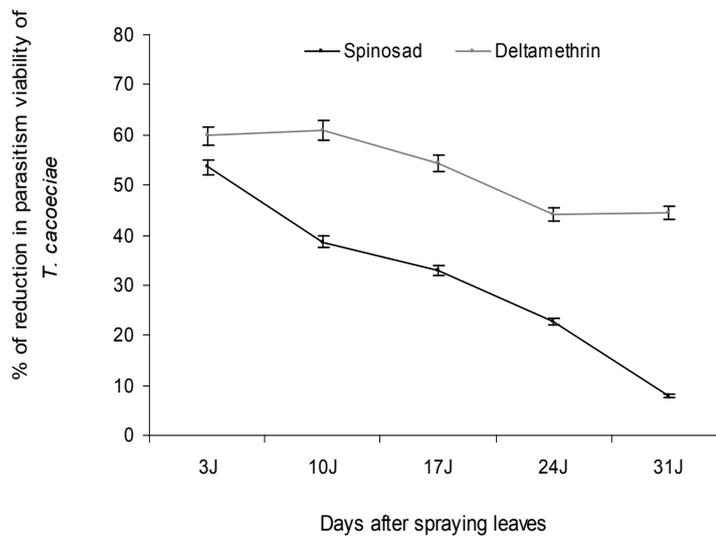


Figure 2. Reduction in parasitism viability (%) of *T. cacoeciae* at days after spraying leaves.

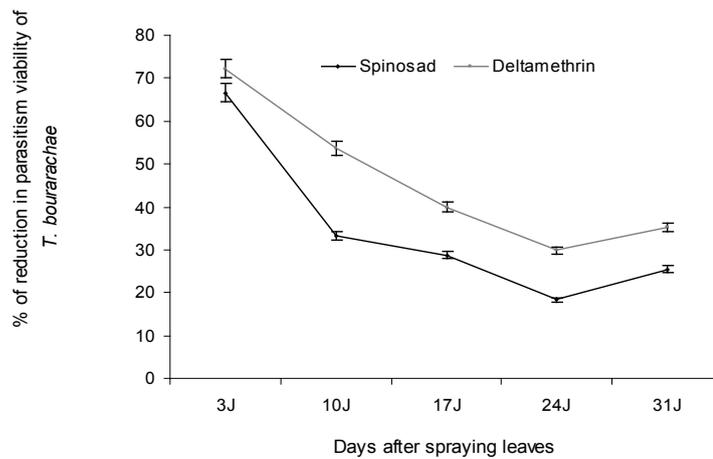


Figure 3. Reduction in parasitism viability (%) of *T. bourarachae* at days after spraying leaves.

However, the effect seemed to differ not only from the spinosad to deltamethrin treatments but also from specie to specie. The deltamethrin treatment reduced parasitism viability of *T. oleae* at 50% even 17 days after parasitoid pupae exposure to residues on leaves. In fact, *T. oleae* displayed a parasitism viability of 80.3% in control assessment, reduced to 40.15% at 17 days after the treatment. Percentage of reduction in parasitism viability of *T. oleae* decreases to 36% on day 24 and to 13% on day 31 (Figure 1).

Spinosad exhibited low toxicity 10 days after exposure. For *T. oleae*, parasitism viability was similar to the control at 78.9% on day 24 and percentage of reduction in parasitism viability was 4.51% (Figure 1). For *T. cacoeciae*, deltamethrin was persisted at 31 days after the treatment showing a negative effect on parasitism viability, reduced to 44% (Figure 2). Also, spinosad exhibited a negative effect on parasitism viability at 17 days after the treatment but 24 and 31 days after exposure to the treatments, reduction in parasitism viability rates decreased to 21% and 7.05% respectively (Figure 2).

For *T. bourarachae*, reductions in parasitism viability curves for exposure time have the same shape for both insecticides. Indeed, reduction in parasitism viability gradually decreases with increasing time after insecticide spraying (Figure 3). Deltamethrin and spinosad exhibited high reduction in parasitism viability of *T. bourarachae* at 3 days after treatment (72.13% and 66.70% respectively) but parasitism viability declined much more in than was the case for *T. oleae* or *T. cacoeciae*. At 31 days after parasitoid pupae exposure to residues on leaves, parasitism viability of *T. bourarachae* was reduced for the deltamethrin treatment at 35.5% and for the spinosad treatment at 25.5% (Figure 3).

4. Discussion

Parasitoids in the genus *Trichogramma* are reported to be excellent indicator species associated with the side effects of pesticides [25]. It was found that insecticides affected *Trichogramma* emergence from host eggs when exposed at different preimaginal stages of development (larval, prepupal, or pupal) [7]. The present study was designed to evaluate three *Trichogramma* species: *T. oleae*, *T. cacoeciae* and *T. bourarachae* for their susceptibility to deltamethrin and spinosad when exposed at pupal stage of development. These parasitoids were candidate to be used in pest management programmes against the second generation of *P. oleae*, as it is especially difficult to be managed with insecticides in most olive areas in Tunisia.

The insecticide concentrations tested were equivalent to those recommended for field application. Side effect evaluation is carried out by measuring parasitism viability rates and adult emergence time of *Trichogramma* species exposed to residues on olive foliage at different time intervals. These measures are important in order to investigate compatibility of *Trichogramma* species with insecticide residues after exposure under semi-field conditions. Most laboratory and field studies have shown that *Trichogramma* wasps are highly susceptible to broad-spectrum insecticides [26]. Consequently, use of insecticides and *Trichogramma* has historically been considered incompatible. The insecticides from the group of Piretroids were commonly described as harmful to beneficial arthropods [27]. According to the IOBC standard methods, deltamethrin is classified as harmful for adults and as slightly harmful for pupae of *Trichogramma* [28]. Similarly, this study demonstrate that exposure to deltamethrin resulted in parasitism viability reduction of the three *Trichogramma* species and showed slightly harmful effect for the parasitoid pupae of *T. oleae* and *T. cacoeciae* ($30\% < E < 79\%$). However, *T. bourarachae* exhibited moderately harmful effect 3 days after pupae exposure to deltamethrin residues on leaves ($80\% < E < 99\%$). Additionally, data show that residues from the deltamethrin treatments were remaining after 24 days. This result indicates that persistence ranged widely, from three to 31 days.

Spinosad, which is obtained from a naturally occurring soil organism, is not considered without risks on beneficials such as predatory insects [29]. However, although spinosad was slightly harmful at the outset of this study ($30\% < E < 79\%$), reduction in parasitism viability decreased after a period of exposure to the treatment. This result is similar to this reported by [12], showing that spinosad degrade quickly following application in the field with residues no longer toxic by 7 to 10 days post-application. However, it appears from our results that susceptibility to spinosad differed from specie to another even after a period of exposure to the treatment.

Overall, the present experiment demonstrated that spinosad was less harmful, based on effect on parasitism viability, than deltamethrin to the three *Trichogramma* species. At the label rates, spinosad was harmless ($E < 30\%$) to *T. oleae* and *T. cacoeciae* and slightly harmful ($30\% < E < 79\%$) to *T. bourarachae* after exposure at 31 days. Spinosad appears to be more compatible with the three *Trichogramma* species than deltamethrin which exhibited moderately harmful effects to *T. bourarachae* ($80\% < E < 99\%$).

Differences regarding susceptibility to the two insecticides show that *T. cacoeciae* was more susceptible to spinosad than *T. oleae*. Also, *T. bourarachae* was more susceptible to the insecticides than *T. oleae* and *T. cacoeciae* with a decrease in parasitism viability when compared to the control. Besides longevity and fecundity that are key attribute for the quality of commercially available *Trichogramma*, the result of this study is important in biocontrol programs where the choice of less susceptible specie to insecticide can be critical.

Although some studies showed that pre-imaginal stages developing within host eggs appear to be protected from many insecticides, [26] reported that spinosad and other piretroids adversely affected *Trichogramma* emergence from host eggs when exposed at different pre-imaginal stages of development (larval, pre-pupal, or pupal). In addition, our results indicate that *Trichogramma* pupa is susceptible to residues of spinosad as well deltamethrin weathered on olive leaves for up to 3 at 31 days.

Adult emergence time (Pupa-adult development time) appeared to be increased for 1 to 2 days when *Trichogramma* pupa were exposed to insecticide residues at the shortest period after treatment, whereas, exposure at longer periods after the treatment seemed to eliminate this effect, especially, for *T. oleae* and *T. cacoeciae*. Thus, the increased length of the adult emergence time is likely related to the susceptibility of *Trichogramma* species in their response to insecticide residues at the different exposure periods.

Residue analysis provides a measure of the nature and level of any chemical contamination within the environment and of its persistence. However, it is often difficult to correlate pesticide residues in the environment with effects on fauna and/or ecological processes. Furthermore, when using insecticides, the relationship between the toxicant and the unit of live is greater for smaller organisms [30]. Thus, we were interested to show whether the insect parasitoid has been exposed to direct toxic effect of chemicals and to identify its potential use for future biological control programmes in the environment.

For the pesticide risk assessment, the exposure characterization describes the actual contact of pesticides and its residue with the parasitoid and the objective was to describe exposure in terms of live, susceptibility and parasitism viability of *Trichogramma* species. This study show that exposure of *Trichogramma* species to spinosad is probably only of concern for 3 - 15 days after spraying. However, exposure to deltamethrin residues is likely to be persistent effect on the parasitoid.

Even experiments were carried out under laboratory conditions where the parasitoid was probably submitted to the highest insecticide pressure, *Trichogramma* release period might be expected, avoiding harmful insecticide residue, to make a better contribution to *P. oleae* second generation control. More generally this can suggests that an insecticide-free environment needs to be considered to ensure maximum parasitism viability of *Trichogramma* in the field. Thus, under field conditions, adults' emergency from parasitized pupa the time of release, would not be affected by the residual activity of the insecticides if *Trichogramma* emergence occurred 24 days after treatment (case of *T. oleae* and *T. cacoeciae*) and 31 days after treatment (case of *T. bourarachae*).

Nevertheless, this prediction requires validation by field studies for example under particular dry conditions that may result in greater persistence of residues [14].

Releasing native species is important since these species have the advantage that they are already adapted to the local environmental conditions of olive tree. However, the susceptibility of a *Trichogramma* parasitoid to the insecticide may be influenced by the ecological attributes of the introduced species [31]. This is, in fact, one of the reasons that make the introduction of *Trichogramma* species to the field conditions an interesting topic of study in the broader area of insecticide effects. This laboratory study must be validated with a field one studying the interactions between biotic performance of *Trichogramma* specie with the weather, foliage, selective forms of application and dosages of insecticides, to obtain an adequate relationship between effectiveness of treatment with the parasitoid fecundity. Accordingly, further studies are currently under way to gain better insights on *Trichogramma* species effectiveness for biological control programs.

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