© by PSP Volume 26 – No. 3/2017, pages 2436-2445 Fresenius Environmental Bulletin

TOXIC EFFECTS OF PYRETHROIDS LAMBDA-CYHALOTHRIN AND ALPHA-CYPERMETHRIN ON PEST *ARCHIPS ROSANA* (LEPIDOPTERA: TORTRICIDAE) AND ITS COMMON PARASITOID

Mitat Aydogdu*, Fulya D Gokalp, Utku Güner

Trakya University, Science Faculty, Biology Department, 22030 Edirne / Turkey

ABSTRACT

In the present study, we aimed to identify the effective concentrations of the insecticides Lambda-cyhalothrin (LCT) and Alpha-cypermethrin (CYP) on different live stages of *Archips rosana* (Linnaeus, 1758), using their commercial forms, Tekvando 5EC (LCT) and Super Takimethrin 100 EC (CYP), as test substances at the field recommended concentration and at 10^1, 10^2, 10^3,10^4, 10^5, 10^6 fold diluted concentrations of the recommended concentration. We found that the LC_{50} concentrations of LCT for larvae, adult (24 h) and pupae forms (7 days) were 1.162 µM, 0.919 µM and 0.012 µM, respectively. The LC_{50} concentrations of CYP for larvae, adult and pupae forms were 1.937, 1.014 and 0.008 µM, respectively. The concentrations that induced over 85% mortality of pupae, although they did not exert a lethal effect on the common parasitoids that developed in the pupae, were 1.10^{-1} µM for LCT and 2.10^{-3} µM for CYP under laboratory conditions. We showed that diluted concentrations of LCT and CYP insecticides still have 80% mortality at the larvae, pupae and adult stages. It is important to study the concentrations that are effective on pests, but not on parasitoids, which are used in biological control. Therefore, this approach may be helpful for integrated pest management programmes.

KEYWORDS:
Alpha-cypermethrin, *Archips rosana*, Lambda-cyhalothrin, parasitoid, pest-control

INTRODUCTION

In many countries, the rates of pesticides use in agricultural areas are continuing to rise. In order to increase crop yield in agriculture, as the use of pesticide increases so does the accumulation of pesticide residues in soil and water, and it is possible to reach other living organisms. Pesticides contaminate living organisms, either directly or by their accumulation in food chains. They can be mutagenic, and may damage the genetic structure of these organisms. Therefore, to avoid or minimise the exposure of living organisms, pesticides use should be reduced, or provide biological control [1].

Beneficial arthropods in agriculture, mainly natural enemies and pollinators, are increasingly important both economically and environmentally. In conventional cropping systems, application of chemical pesticides often leads to extinction of natural enemy populations, either through the direct effect of the pesticides or through the disappearance of the food/host (i.e. the pests) to sustain natural enemies. By contrast, an increase in sustainability and profitability of using a given pesticide is directly related to its selectivity, i.e. killing the targeted pest(s) and preserving the beneficial arthropods (which could provide ecological services, such as biological control and pollination). Sustaining the efficacy of natural enemies against pests is a key component in IPM programmes and organic farming, and thus pesticide applications should mitigate the possible harmful effects to these beneficial arthropods [2-3].

Insecticides are an important constituent of our daily life, from preservation of food materials to insect pest management. Pyrethroids are a group of pesticides that have been widely used in recent years, and are currently among the major insecticides applied against Lepidoptera and other pests [4]. Lambda-cyhalothrin (LCT) and Alpha-cypermethrin (CYP) are synthetic type II pyrethroids that are used for insect control [2]. Synthetic pyrethroids are neuropoisons that act on the axons in the peripheral and central nervous systems, altering the axonic sodium channels and allowing excessive ion entrance, thereby causing abnormal nervous activity and eventually paralysis [5]. It has been reported that these pyrethroids have cytotoxic and genotoxic effects on non-target organisms. LCT is highly toxic to fish, aquatic arthropods and honeybees [6] the majority of existing genotoxicity studies indicate that LCT has an effect on aquatic organisms and mammals [7-10], while CYP has a genotoxic effect in human lymphocytes [11-12]. These toxic effects mean that

it is important to reduce the amount of the pesticides use in agriculture. Therefore, many previous studies assessed the effectiveness of pesticides on target organisms in integrated pest management (IPM) programmes. It was found that LCT was highly toxic to adult *Chrysoperla carnea* (Neuroptera: Chrysopidea). LCT and CYP were classified as moderately harmful, according to the mortality percentages [13]. CYP is highly toxic to egg and larvae of *C. externa* (Neuroptera: Chrysopidea) [14] and slightly harmful [15] to *Chrysoperla carnea* larvae. Lower concentrations of CYP inhibited larval growth of *Rachiplusia nu* (Lepidoptera: Noctuidae) by 75% to 98% [16].

Application of insecticides at high concentrations, or in large amounts, also affects beneficial organisms, such as parasitoids, which could be used in biological control in agriculture. Therefore, some studies have indicated that only those pesticides that are the most selective, and which have no adverse effects on beneficial organisms, should be used [17-18]. Other studies have attempted to identify the pesticides, and useful concentrations, which are effective on pests, but not on beneficial organisms [13]. In the present study, we selected two insecticides that are used extensively to control pests in orchards, and tested their toxicity against the larvae, pupae and adult of *A. rosana* under laboratory conditions.

The European leaf roller (ELR) Rose Tortrix *A. rosana* is a moth of the family Tortricidae (Lepidoptera). *A. rosana* is a species that is native to the Palearctic region, but it is found all over the world, with the exception of the Far East and Siberia. The ELR is a primary, or sometimes secondary, pest in orchards, depending on the time and the location. Tortricidae family plays an important role in plant protection, due to its large number of harmful species and frequent occurrence in different cultivations. In recent years, observations conducted in orchards have shown that the population and economic importance of this phytophagous species is increasing, which often makes the use of chemical control necessary. However, tortricids are attended by a large number of natural enemies, which is why much attention is given to the preparation of programmes to control them by means of selective treatment [19-20]. The ELR pest is univoltine, and its life cycle starts with the larvae hatching from overwintering eggs in late February. The larval period is approximately 6–8 weeks in duration. Pupation occurs within the rolled and webbed leaves. In Southern Turkey, pupation occurs at the end of May and in early June. Adult emergence begins in the second week of June, and usually continues until mid- August; adults live for 2–4 weeks. The pest feeds on a large variety of shrubs and trees [21-22].

Large amounts of the pyrethroid insecticides LCT and CYP are used against *A.rosana*, which is one of the major orchard pests in the Thrace region in the north west of Turkey. Its short life-span makes its control rather difficult, if not monitored regularly. Effective doses are important for selection of insecticides, which must be tested at different life-stages of insects. The life-cycle times of larvae, pupae and adult forms are known, so it may be useful to know the effective concentrations at these different stages. Moreover, this knowledge may be useful in decreasing the use of insecticides in agriculture. In the present study, we aimed to investigate both lethal/effective concentrations of LCT and CYP on different life stages of *A.rosana*, as well as the effects of insecticides on the parasitoid *Itoplectis maculator* (Fabricius, 1775). *I. maculator* is a specific and dominant endoparasitoid of *A. rosana* [23]. *I. maculator* is a potential biological control agent. Therefore, its relationship with insecticides should be revealed.

MATERIALS AND METHODS

Study Area. The toxicity of LCT and CYP was investigated against last instar larvae, adult and pupae of *A. rosana* under laboratory conditions. Larvae and pupae were collected from almond, apple, plum and cherry trees in the vicinity of Edirne (north-west Turkey) in the early mornings, ensuring to minimize hand contact with the larvae and pupae. The collections were made from locations of Edirne region that were chemical free areas and not used for agricultural purposes. We used 10 larvae, pupae and adults of *A. rosana* in our experiments, which we replicated three times. Mortality of larvae, pupae and adults were determined on the basis of completion of their life cycles as each stage, and mortality rates of larvae and pupae were recorded every 24 h until the organisms reached the adult stage. We used the data collected to calculate the mortality percentages for each development stage. We obtained the adults from the rearing of unexposed larvae and pupae under laboratory conditions.

Test Pesticides. We used commercial formulations of LCT and CYP as test chemicals. The commercial names, CAS numbers and amount of active substance in the commercial forms are given in Table 1.

We prepared a series of standards of known concentration of ranging from low to high recommended by manufacturer. Exposures of insecticides were expressed as concentrations of the active ingredient. At the recommended concentration, the active ingredient of Tekvando 5EC is 10 µM LCT, while the active ingredient of Super Takimethrin 100EC is 20 µM CYP. We prepared test concentrations of LCT and CYP according to their recommended concentrations;

TABLE 1
Commercial names, formulations, amount of active substances, and classes of pesticides; LCT and CYP

Insecticide name	CAS no	Commercial Name	Formulation	Active substance amount	Dose ml/da	Class
Alpha-cypermethrin	67375-30-8	Super Takimethrin 100 EC	EC	100 g/l	20	Synthetic pyrethroid
Lambda-cyhalothrin	68085-85-8	Tekvando 5 EC	EC	50 g/l	20	Synthetic Pyrethroid

10, 1, 1.10^{-1}, 1.10^{-2}, 1.10^{-3}, 1.10^{-4} , $1.10^{-5}$and 1.10^{-6} µM LCT, and 20, 2, 2.10^{-1}, 2.10^{-2}, 2.10^{-3}, 2.10^{-4}, 2.10^{-5} and 2.10^{-6} µM CYP, and tested these concentrations on larvae, pupae and adult forms of *A. rosana*. We freshly prepared the test solutions in distilled water, and used leaves exposed with distilled water as a control. Then lethal concentration for 50% of the population was estimated (LC_{50}).

Larvae Bioassay. Last instar larvae and pupae of the pest were collected from selected study area. We fed *A. rosana* larvae on an artificial diet in a laboratory in petri plates [24]. We maintained each individual larvae in large Petri plates (10 cm diameter) containing a 50% solution of diluted honey embedded in cotton pieces, which we used as the food source during the experiments [25-26] and cherry leaves, under laboratory conditions at 25 ± 2°C, 70% RH and a photoperiod of 16:8 h (L:D). We prepared laboratory cultures using a standard leaf disc bioassay method [27-28]. We collected cherry leaves from unsprayed plants, washed and air-dried, and made 5 cm diameter leaf discs, which we dipped in a test solution for 10 s and allowed to dry at ambient temperature for approximately 20–30 min. We placed the leaves in a single disposable petri plate of 6 cm in diameter, and recorded mortality rates at 24, 48, 72 and 96 hours. At the end of the recording period, we calculated the lethal concentrations for 50% (LC_{50}) and 90% (LC_{90}) of the populations for each insecticide, according to 24-h mortality data. We determined mortality on the basis of life-cycle completion in the pupae and adult stages.

Pupae Bioassay. We dipped *A.rosana* pupae in test solutions for 1 s, placed them in clean petri plates with clean leaves, and kept them under identical laboratory conditions. The mortality rate was recorded at 7, 12 and 15 days, and LC_{50} and LC_{90} values were calculated according to 7 days' mortality data. We opened unmatured death pupae to determine the presence of cephalic structures of parasitoids, in the event of death due to the effect of insecticide or to parasitism [7]. We therefore excluded death pupae due to parasitism from the data.

Parasitoid Bioassay. It was stated that 25% of larvae and pupae of *A. rosana* were attacked by mostly seen parasitoid *I. maculator* [23]. Pupae collected from selected study area, were exposed to different concentrations of LCT and CYP and observed them until adult stage. We observed parasitoid growth until the parasitoid emerged from Tortricidae pupae, recorded the number of parasitoids that completed life cycle stages until reaching the adult stage. The average numbers of surviving parasitoids were observed with the exposed and unexposed pupae at the end of day 7.

Adult Exposure. We allowed the material (larvae and pupae) collected from unexposed trees to grow to adult forms in glass cages containing a 50% solution of diluted honey embedded in cotton pieces and fresh, clean leaves from cherry trees. After growth, we removed the lepidopteron adults from petri plates with the aid of a vacuum hose and placed them in new petri plates. After obtaining adult forms of *A. rosana*, we prepared for them the exposures that had been used with the larvae forms. We observed adult mortality rates at 24, 48, 72 and 96 h, and calculated LC_{50} and LC_{90} concentrations according to 24-h mortality data.

Statistical Analysis. We analysed the percentage mortality data for all exposures using one-way analysis of variance, and calculated LC_{50} and LC_{90} values by probit analysis, using the SPSS programmed (version 15.0). We evaluated the significances of differences between groups at the level of $P<0.05$, and recorded the differences as *$P<0.05$, **$P<0.01$ ***$P<0.001$ in tables.

We based the criterion for evaluating the efficiency of insecticides on insects at the threshold of 80% mortality in laboratory tests with a local (field) population. This criterion of efficiency is required by the Turkish Ministry of Agriculture for registration of an insecticide for agricultural use in Turkey. Therefore, we considered an insecticide to be effective if it resulted in a mortality rate that was equal to or greater than 80%. We calculated the average number of surviving parasitoids in 10 pupae of three replicates and compared the vitality of parasitoids in the exposed groups to that of the control group using a T-test. We evaluated the significances of differences between groups at the level of $p<0.05$.

TABLE 2
Mortality of larvae and adult forms of *A. rosana* after 24, 48, 72 h and pupae after 7, 12, 15 days following single exposure of different concentrations of LCT

Life stages of *A. rosana*	Applied concentrations of Lambda-cyhalothrin	Mortality % ± S.E. Times after application			
		24 h	**48 h**	**72h**	**96 h**
Larvae	-control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	10 µM (r.d.)	100.0±0.0***	100.0±0.0***	100.0±0.0***	100.0±0.0***
	1 µM	40.0±6.12***	55.0±5.0***	75.0±0.0***	100.0±0.0***
	1.10^{-1} µM	35.0±6.12***	65.0±6.12***	80.0±5.0***	80.0±5.0***
	1.10^{-2} µM	10.0.±6.12	35.0±6.12***	50.0±7.9***	60.0±6.1***
	1.10^{-3} µM	0.0±0.0	0.0±0.0	15.0±6.1	15.0±6.1*
	1.10^{-4} µM	0.0±0.0	0.0±0.0	5.0±5.0	5.0±5.0
	1.10^{-5} µM	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		24 h	**48 h**	**72 h**	**96 h**
Adult	-control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	10 µM (r.d.)	100.0±0.0***	100.0±0.0***	100.0±0.0***	100.0±0.0***
	1 µM	60.0±10.0***	85.0±6.1***	95.0±5.0***	100.0±0.0***
	1.10^{-1} µM	5.0±5.0	25.0±7.9*	40.0±6.1***	75.0±11.18***
	1.10^{-2} µM	0.0±0.0	30.0±9.35**	45.0±5.0***	60.0±6.12***
	1.10^{-3} µM	0.0±0.0	5.0±5.0	40.0±6.1***	60.0±6.12***
	1.10^{-4} µM	0.0±0.0	5.0±5.0	25.0±0.0***	35.0±6.12***
	1.10^{-5} µM	0.0±0.0	5.0±5.0	10.0±6.1	5.0±5.0
	1.10^{-6} µM	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		7 days	**12 days**	**15 days**	
Pupae	-control	0.0±0.0	0.0±0.0	0.0±0.0	
	10 µM (r.d.)	100.0±0.0***	100.0±0.0***	100.0±0.0***	
	1 µM	100.0±0.0***	100.0±0.0***	100.0±0.0***	
	1.10^{-1} µM	85.0±6.12***	100.0±0.0***	100.0±0.0***	
	1.10^{-2} µM	85.0±10.0***	100.0±0.0***	100.0±0.0***	
	1.10^{-3} µM	95.0±5.0***	100.0±0.0***	100.0±0.0***	
	1.10^{-4} µM	0.0±0.0	95.0±5.0***	100.0±0.0***	
	1.10^{-5} µM	0.0±0.0	95.0±5.0***	100.0±0.0***	
	1.10^{-6} µM	0.0±0.0	95.0±5.0***	100.0±0.0***	

Dunnett t test (*P<0.05; ** P<0.01; *** P<0.001)

RESULTS

The results we obtained after exposure of LCT and CYP at the larvae, pupae and adult stages are shown in Tables 2 and 3.

Larval Stage Exposure. The results showed that 10^{1}-10^{3} fold diluted concentrations of the recommended concentrations of LCT and CYP (1, 1.10^{-1} and 1.10^{-2} µM, and 2, 2.10^{-1}, 2.10^{-2} and 2.10^{-3} µM, respectively) caused significant larval mortality at different time periods. However, the lowest concentration that caused larval mortality over 80% was 1.10^{-1} µM for LCT and 2 µM for CYP after 72 h. We observed that the pesticides were significantly effective at lower concentrations at 72 h and 96 h. The respective LC_{50} and LC_{90} concentrations of LCT (larval stage) were 1.162 (0.747–1.935) µM and 2.134 (1.488–4.132) µM at 24 h. These concentrations are lower than the recommended concentration in agricultural areas. In addition, the LC_{50} and LC_{90} concentrations (larval stage) of CYP were 1.937 (1.413–3.100) µM and 3.625 (2.649–6.079) µM, respectively, at 24 h (Table 4). The significance of larval mortality increased with time. The results of the 1.10^{-6} µM LCT and 2.10^{-6} µM CYP larvae exposures are not shown in the Tables.

Adult Stage Exposure. The results we observed after adult exposure showed that mortality increased. The lowest concentration that caused a rate of adult mortality over 80% was 1 µM at 48 h for LCT, and 2.10^{-1} µM at 72 h for CYP. CYP was more effective than LCT at the adult stage of *A. rosana*. We found LC_{50} and LC_{90} concentrations of LCT (adult stage) of 0.919 (0.756–1.142) µM and 1.356 (1.135–1.796) µM, respectively, at 24 h (Table 4). This concentration was lower than the recommended concentration in agricultural areas. In addition, we observed LC_{50} and LC_{90} concentrations of CYP (adult stage) of 1.014 (0.554–2.253) µM and 1.967 (1.262–4.618) µM, respectively, at 24 h (Table 4).

Pupae Stage Exposure. We observed the mortality of pupae after a single-dose exposure after 7, 12 and 15 days. Although the control specimens remained alive for 15 days, exposed pupae samples of A. rosana are very sensitive. The lowest concentration that caused pupae mortality of over

80% was 1.10-3 µM for LCT and 2.10-3 µM for CYP at 7 days. The LC50 and LC90 concentrations of LCT (pupae stage) were 0.012 µM and 0.137 µM, respectively, at 24 h (Table 4). This concentration was lower than the recommended concentration in agricultural areas. In addition, the LC50 and LC90 concentrations of CYP (pupae stage) were 0.008 µM and 0.152 µM, respectively, at 24 h (Table 4). The results of the 2.10-6 µM CYP pupae exposure are not shown in Table 3.

Parasitoid Vitality. We also calculated the average number of surviving parasitoids to show the concentration that has a lethal effect on pests, but not on parasitoids, as the parasitoids are beneficial organisms and are used in biological control. *Itoplectis maculator* (Fabricus, 1775) (Ichneumonidae, Hymenoptera) is the most common parasitoid, followed by *Brachymera tibialis* (Walker, 1834) (Hymenoptera, Chalcididae) and *Eumea linearicornis* (Zett, 1844) (Diptera, Tachinidae). In the present study, *I. maculator* was the most observed parasitoid. The average number of surviving parasitoids that were developed in pupae in 7 days is given in Table 5. The average number of surviving parasitoids significantly decreased from 2.6 to 0.2 after 10 µM LCT and 20 µM CYP (recommended concentrations) exposures (Table5).

TABLE 3
Mortality of larvae and adult forms of *A. rosana* after 24, 48, 72 h and pupae after 7, 12, 15 days following single exposure of different concentrations of CYP

Life stages of *A. rosana*	Applied concentrations of Alpha-cypermethrin	Mortality % ± S.E. Times after application			
		24 h	**48 h**	**72 h**	**96 h**
Larvae	-control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	20 µM (r.d.)	100.0±0.0***	100.0±0.0***	100.0±0.0***	100.0±0.0***
	2 µM	50.0±7.9***	65±6.1***	85±10.0***	90.0±6.1***
	2.10^{-1} µM	25.0±0.0***	40±6.12***	55±5.0***	80.0±12.2***
	2.10^{-2} µM	5.0±5.0	30.0±5.0***	35±6.1***	35.0±6.1***
	2.10^{-3} µM	5.0±5.0	10.0±6.1	15.0±10.0	60.0±10.0***
	2.10^{-4} µM	5.0±5.0	5.0±5.0	5.0±5.0	10.0±6.1
	2.10^{-5} µM	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		24 h	**48 h**	**72 h**	**96 h**
Adult	-control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	20 µM (r.d.)	100.0±0.0***	100.0±0.0***	100.0±0.0***	100.0±0.0***
	2 µM	100.0±0***	100.0±0***	100.0±0***	100.0±0.0***
	2.10^{-1} µM	20±9.4*	55±9.4***	80±5***	100.0±0.0***
	2.10^{-2} µM	25±7.9**	50±7.9***	60±10***	85±22.36***
	2.10^{-3} µM	5±5	25±7.9*	55±5***	55±41.09***
	2.10^{-4} µM	0±0	15±10	35±6.1***	50±35.35**
	2.10^{-5} µM	0±0	0.0±0	0±0	0.0±0.0
	2.10^{-6} µM	0±0	0±0	0±0	0.0±0.0
		7 days	**12 days**	**15 days**	
Pupae	-control	0.0±0.0	0.0±0.0	0.0±0.0	
	20 µM (r.d.)	100.0±0.0***	100.0±0.0***	100.0±0.0***	
	2 µM	***100.0±0.0	***100.0±0.0	***100.0±0	
	2.10^{-1} µM	***100.0±0.0	***100.0±0.0	***100.0±0	
	2.10^{-2} µM	***100.0±0.0	***100.0±0.0	***100.0±0	
	2.10^{-3} µM	***90±10	***100.0±0.0	***100.0±0	
	2.10^{-4} µM	***70±9.35	***100.0±0.0	***100.0±0	
	2.10^{-5} µM	***35±6.2	***70±12.24	***100.0±0	

Dunnett t test (*P<0.05; ** P<0.01; *** P<0.001)

TABLE 4
LC_{50} and LC_{90} values of LCT and CYP for *A. rosana* at 24 hours for larvae and adult and 7 days for pupae

Insecticide	Stage	LC_{50} µM(lower-upper bound)	LC_{90} µM(lower-upper bound)
Lambda-cyhalothrin	Larvae	1.162(0.747-1.935)	2.134(1.488-4.132)
	Adult	0.919(0.756-1.142)	1.356(1.135-1.796)
	Pupae	0.012(*)	0.137(*)
Alpha-cypermethrin	Larvae	1.937(1.413-3.100)	3.625(2.649-6.079)
	Adult	1.014(0.554-2.253)	1.967(1.262-4.618)
	Pupae	0.008(*)	0.152(*)

* Lower and upper bounds of confidence limits were not calculated.

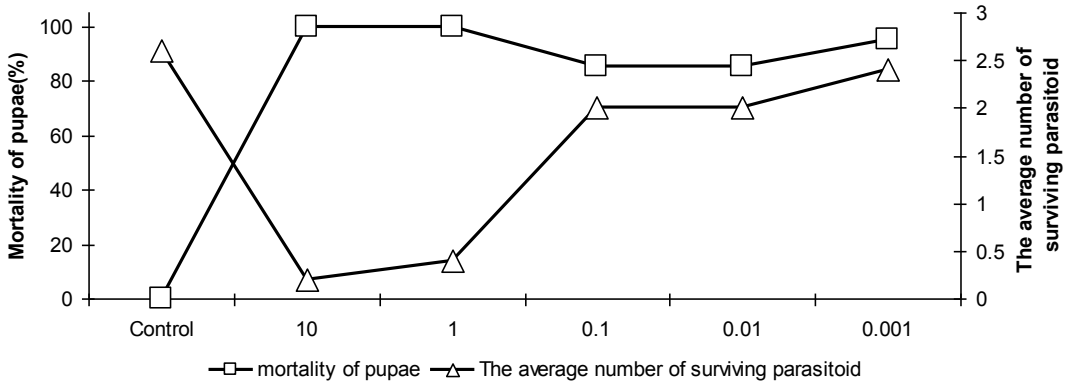

FIGURE 1
The figure shows average number of surviving parasitoid after exposure to LCT at different concentrations (concentration as µM)

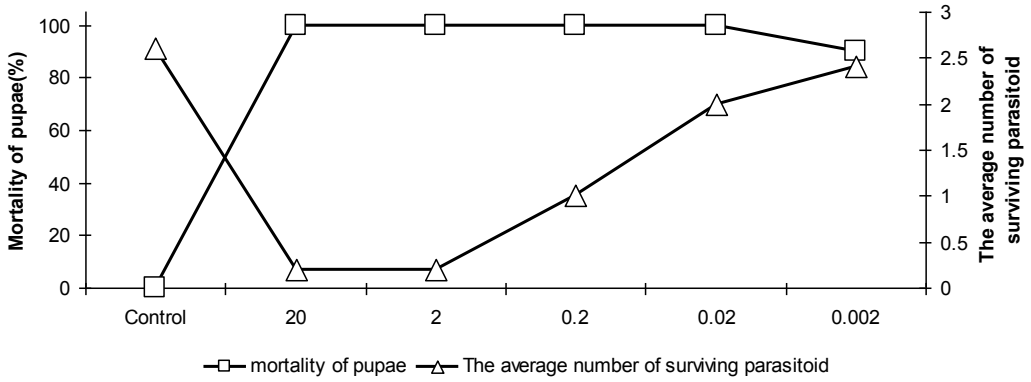

FIGURE 2
The figure shows average number of surviving parasitoid after exposure to CYP at different concentrations (concentration as µM)

TABLE 5
The average number of surviving parasitoids at the end of 7 days (parasitoid/10 pupae)

Lambda-cyhalothrin		Alpha-cypermethrin	
Control	2.6±0.2	Control	2.6±0.24
10 µM	***0.2±0.2	20 µM	***0.2±0.2
1 µM	***0.4±0.24	2 µM	***0.2±0.2
1.10^{-1} µM	2.0±0.31	2.10^{-1} µM	*1.0±0.44
1.10^{-2} µM	2.0±0.31	2.10^{-2} µM	2.0±0.31
1.10^{-3} µM	2.4±0.4	2.10^{-3} µM	2.4±0.4

Figures 1 and 2 show the mortality of pupae and the average number of surviving parasitoids after different concentrations of LCT and CYP exposure at 24 h. An LCT exposure of 1.10^{-1} µM induced 85% pupae mortality, while the average number of surviving parasitoids, which was two, was not significantly different from the control group (Fig 1). Moreover, we obtained similar results with CYP exposure, in that 2.10^{-2} µM CYP exposure induced 90% pupae mortality, while the average number of surviving parasitoids, which was two, was not significantly different from the control group (Fig 2).

DISCUSSION

In the present study, we tested the effectiveness of different concentrations of LCT and CYP on larvae, pupae and adult forms of the pest *A. rosana*. The results showed that LCT and CYP had an effect, even at low concentrations. The life cycle and biology of *A. rosana* is well known; it occurs between March and June in the Thrace region of Turkey [22, 28]. Different life stages of insects may show variable sensitivity to insecticides. One of the aims of the present study was to determine the effect of different

concentrations of LCT and CYP on different life cycle stages; larvae, pupae and adult. We hoped that the results might provide evidence to support the importance of reduced insecticide applications, and, in turn, a decrease in the release of pesticides into the environment. According to the concepts considered within the IPM paradigm, an assessment of the biological effects of pesticides on target organisms is of great relevance for the minimisation of treatment thresholds and the assessment of efficiency of new pesticides [16]. Moreover, in addition to mortality, the sub-lethal effects could also contribute to the reduction of pest population levels. The selection of highly efficient and selective compounds has usually been associated with the study of the worst possible scenarios by means of laboratory toxicity tests; the strategy was undertaken with beneficial organisms by the International Organization for Biological Control [18, 29]. In the present study, we observed a LC_{90} concentration of LCT (at larval stage) of 2.134 (1.488–4.132) µM after 24 h in laboratory conditions. The recommended concentration that is offered in application of LCT (commercial formulation: Tekvando 5EC) contains 10 µM active substance in orchard agriculture. Therefore, 5 times diluted concentrations may be sufficient against *A. rosana* in agriculture. The LC_{90} concentration of CYP (at the larval stage) was 3.625 (2.649–6.079) µM after 24 h in laboratory conditions. The field recommended concentration that is offered in application of CYP (commercial formulation: Super Takimethrin 100EC) contains 20 µM of active substance in orchard agriculture. Therefore, 6 times diluted concentrations may be sufficient against *A. rosana* in agriculture. However, it is important to indicate that this might only be possible on first application for orchards, as the *A. rosana* that we studied was not a resistant form. The larval stage of *A. rosana* occurs between April and May [23]. Therefore, it may be useful if CYP is applied at lower concentrations of field application doses when it is used against this species in orchards for the first time. Similarly, Rimoldi et al. [16] stated that at tested CYP concentrations (between 10% and 80% dilution concentrations of maximum field recommended concentration) larval mortalities were between 75% and 98% [16]. In the present study, we showed that at 10% and 100% dilution concentrations of CYP, larval mortalities were between 55% and 85% at 72 h. We also showed high effectiveness of LCT and CYP in *A. rosana* control, even at concentrations below the field recommended concentration. Rimoldi et al. [16] indicated that although they observed survivors at the lowest tested concentrations of CYP after larvae exposure to *Rachiplusia nu* (Lepidoptera), mortality rates were high, and it may be desirable to keep the pest population below the economic injury level, according to pest control strategies [16]. Other studies have also reported high mortality rates with pyrethroid on Lepidoptera species [30-31].

In the present study, in lower concentrations of the recommended concentration (1.10-1 µM), mortality ratios of larvae reached over %80 and %85 at 72th hour for LCT and CYP respectively. Rimoldi et al. [16] observed the same, in that larvae exposed to CYP took longer to die than larvae exposed to methoxyfenozide. Organisms exposed to cypermethrin consumed very little of the treated diet during the first 24 h of testing. In this way, the reduction of larval weight could be attributed to the reduction of the body fat because larvae consume their reserves (mainly lipids, proteins and carbohydrates) and do not feed because of repellence (anti-feeding effect) and/or disorders at the toxicant modifying the feeding behavior [16]. It was reported that in laboratory observations, *Rhagoletis indifference* (Diptera: Tephritidae) spent the least amount of time on cherries treated with zeta-cypermethrin, possibly because of its toxicity and irritant effects [32].

In our study, we showed that mortality increases with time. This was obvious after exposure to more diluted concentration for both pyrethroid insecticides. Similarly, Sallam et al. [33] showed that the mortality percentage of *Rhopalosiphum padi* (L.) and *Metopolophium dirhodum* (Wlk.) (cereal aphids) increased with time after lambda-cyhalothrin (Karate 9.4 % S.C) exposure at concentrations between 0.3 and 10 ppm. The mortality percentages of *R. padi* were 19.3–86.7 % at 24 h, 33.3–100% at 48 h and 41.6–100% at 72 hours, while the mortality percentages of *M. dirhodum* were 26.7–100 % at 24 h, 39.2–100% at 48 h and 62.5–100% at 72 h [33].

Ahmad et al. [34] studied insecticide mixtures against resistant populations *Spodoptera litura* (Lepidoptera: Noctuidae) [28, 34]. They stated that after a 72-h exposure. In our study, we found that the LC50 doses of CYP after 24 and 72 h were 1.937 (1.413–3.100) and 0.046 (0.019–0.116), respectively, for larvae, and 1.014 (0.554–2.253) and 0.07(0.009–1.372) respectively, for adults. The difference in the results may be due to exposure of resistant and sensitive forms of different species of pests.

We showed that parasitoids, which are beneficial organisms in fighting pests, were also affected by exposure to LCT and CYP insecticides. It has been stated that *I. maculator* (Ichneumonidae, Hymenoptera) is a specific and dominant endoparasitoid of *A. rosana* [23]. Parasitoids from the superfamily Ichneumonoidea (Braconidae and Ichneumonidae) are the most effective endoparasitoid of pest, and the dominant species was *Itoplectis maculator* (Fabricius, 1775) [1]. In the present study, we most frequently observed the parasitoid *I. maculator*.

In the aforementioned study, Aydogdu and

Güner [1] assessed the effects of commercial formulations of five different insecticides (Alpha-cypermethrin, Diazinon, Dichlorvos, Deltamethrin, Lambda-cyhalothrin) used in orchards on the 24-h mortality rates of the parasitoid *I. maculator* under laboratory conditions. The study showed that LT/KDT_{50} values for LCT and CYP for adult forms were 2.63 and 2.54, respectively. The author stated that experimental bees exposed to Diazinon, Dichlorvos and Deltamethrin had died at the end of h 8, while those exposed to Lambda-cyhalothrin and Alpha-cypermethrin died at the end of h 24. This means that LCT and CYP provide a chance to live longer than do the other three insecticides [1]. In addition, the author also stated that *I. maculator* is a potential biological control agent that could be used in orchards, so its relationship with insecticides should be described.

We found that, at recommended concentration exposures, the parasitoids could not develop inside the pupae. However, in diluted concentrations, the insecticide was effective on the pupae of pests, but not on parasitoids. The results showed that, following exposure of pupae to 1.10^{-1} µM LCT, while the pupae mortality was 85%, the average number of surviving parasitoids was two, which was not significantly different from the control group (Fig 1). We also observed a similar result with CYP, in that 2.10^{-2} µM CYP exposure increased the average number of surviving parasitoids, although it protected 90% of pupae from mortality. This result also might be useful for IPM programmes. The data that have been obtained to date may provide evidence in support of decreasing the number and concentration of applications, as well as the release of these pesticides into the environment. If the time spans of pupae formations in various localities are known, it may be useful to fight with insecticides as they are sensitive, but also protect the beneficial organisms that are parasitoids.

The aim of an IPM is to minimize pesticide use, evaluate all types of pest control opportunities and make use of natural enemies of pest organisms as much as possible [35-36]. Natural enemies are the key component of IPM, and they are often recommended as the first line of defence in an IPM programme [37]. The most crucial requirement for pesticides is that they be compatible with biological control. It has been stated that the only selected pesticides that should be used are those that are the most selective and which have no adverse effects on beneficial organisms [17-18]. It has been recommended that conventional pesticides must be used with appropriate formulations in the right concentration and at the optimum time of intervention following proper application methods to avoid damaged by natural enemies [13].

In the present study, LCT and CYP induced high mortality and important sub-lethal effects at concentrations below field recommended concentrations. Therefore, future studies should include the assessment of these concentrations in semi-field and field conditions towards diminishing recommended application doses. It might be better to use both low doses of insecticides and biological control agents simultaneously in agriculture. Thus, while biological control continues, conversely, the amount of pesticide use may be reduced.

REFERENCES

[1] Aydoğdu, M. and Güner, U. (2012) Effects of 5 different insecticides on mortality of the Leafroller parazitoid *Itoplectis maculator* (Fabricius, 1775) (Ichneumonidae, Hymenoptera). Türkiye Entomoloji Bülteni 2(4): 243-249.

[2] Biondi, A., Mommaerts, V., Smagghe ,G., Viñuela, E., Zappalà, L. and Desneux, N. (2012) The non target impact of spinosyns on beneficial arthropods. Pest Management Science 68(12): 1523-1536.

[3] Desneux, N., Decourtye, A. and Delpuech J.M. (2007) The Sublethal Effects of Pesticides on Beneficial Arthropods. Annual Review of Entomology 52(1): 81-106.

[4] Pietrantonio, P.V., T. A. Junek, R. Parker, D. Mott, K. Siders, N. Troxclair, J. Vargas-Camplis, J. K. Westbrook, and Vassiliou, V.A. (2007) Detection and Evolution of Resistance to the Pyrethroid Cypermethrin in *Helicoverpa zea* (Lepidoptera, Noctuidae) Populations in Texas. *Environmental Entomology* 36(5):1174-88.

[5] Stenersen, J. (2004) Chemical pesticides mode of action and toxicology, CRC press.

[6] Anonymous, (1990) World Health Organisation. Environmental Health Criteria 99: Cyhalothrin. 8.

[7] Chaudhari, S. (2013) Biometrial Measurments of Life Stages of *Senometopia illota* Curran (Diptera: Tachinidae) A Larval Pupal Parasitoid on *Helicoverpa armigra* Hübner. International Journal of Bioassays 2(6): 850-857.

[8] Muranlı, F.D.G. (2009) Genotoxic and cytotoxic effects of a pyrethroid insecticide Lambda-cyhalothrin on human peripheral blood lymphocytes investigated by chromosome aberration and flow cytometry assays. Fresen. Environ. Bull. 18(9a):1758-1763.

[9] Fetoui, H., Feki ,A., Ben Salah, G., Kamoun, H., Fakhfakh, F. and Gdoura, R. (2013) Exposure to lambda-cyhalothrin, a synthetic pyrethroid, increases reactive oxygen species production and induces genotoxicity in rat peripheral blood. Toxicol Ind Health.

[10] Fahmy, M.A. and Abdalla, E. (2001) Cytogenetic effects induced by the natural pyrethrins and the synthetic lambda cyhalothrin in mice in vivo. Cytologia: International Journal of Cytology 66(2): 139-149.
[11] Kocaman, A.Y., Topaktaş, M, (2009) The in vitro genotoxic effects of a commercial formulationof Alpha-cypermethrin in human peripheral blood lymphocytes. Environmental and Molecular Mutagenesis 50(1): 27-36.
[12] Muranlı, F.D.G. (2013) Genotoxic and cytotoxic evaluation of pyrethroid insecticides λ-cyhalothrin and α-cypermethrin on human blood lymphocyte culture. Bulletin of Environmental Contamination and Toxicology 90(3): 357-363.
[13] Sabry, K. and El-Sayed, A. (2011) Biosafety of a biopesticide and some pesticides used on cotton crop against green lacewing, *Chrysoperla carnea* (Stehens) (Neuroptera: Chrysopidae). Journal of Biopesticides 4(2): 214-218.
[14] Schneider, M., Pineda, P. and Smagghe, G. (2005) Side effects of conventional and non-conventional insecticides on eggs and larvae of *Chrysoperla externa* (Hagen)(Neuroptera: Chrysopidae) in Argentine. Communications in Agricultural and Applied Biological Sciences 71(2): 425-427.
[15] Reddy, G., Divakar, B. (1998) Relative toxicity of nine insecticides to *Bracon kirkpatricki* and *Chrysoperla carnea*. Plant protection Bulletin Faridabad 50(1-4): 9-10.
[16] Rimoldi, F., Fogel, M.N., Schneider, M.I., Ronco, A.E. (2012) Lethal and sublethal effects of cypermethrin and methoxyfenozide on the larvae of *Rachiplusia nu* (Guenee) (Lepidoptera: Noctuidae). Invertebrate Reproduction & Development 56(3): 200-208.
[17] Nasreen, A., Ashfaq, M., Mustafa, G. and Khan, R.R. (2007) Mortality rates of five commercial insecticides on *Chrysoperla carnea* (Stephens)(Chrysopidae: Neuroptera). Pakistan Journal of Agricultural Science 44: 266-271.
[18] Hassan, S. (1989) Testing methodology and the concept of the IOBC/WPRS working group. Pesticides and non-target invertebrates/editor: Paul C. Jepson.
[19] Kot, I. (2007) Parasitic entomofauna of leaf tortricids (Lepidoptera: Tortricidae) occurring in apple orchards. EJPAU 10(1), art.#27.
[20] Aydogdu, M. and Beyarslan, A. (2012) A review of the genus *Ascogaster* Wesmael, 1835 (Hymenoptera, Braconidae, Cheloninae) in Turkey, with a new host record for *Ascogas-terbicarinata* (Herrich-Schãffer, 1838). North-Western Journal of Zoology 8(1): 31-40.
[21] Ulu, O. (1983) İzmir ve Manisa İlleri Çevresi Taş Çekirdekli Meyve Ağaçlarında Zarar Yapan *Archips* (=*Cacoecia* spp.) (Lepidoptera: Tortricidae) Türleri, Tanımları, Konukçuları, Yayılışları ve Kısa Biyolojileri Üzerinde Araştırmalar. Zirai Mücadele Zirai Karantina Genel Müdürlüğü, Bornova Bölge Zirai Mücadele Araştırma Enstitüsü Müdürlüğü Araştırma Eserleri Serisi 45.
[22] Doganlar, O. (2007) Distribution of European Leaf Roller, *Archips rosanus* (L.) (Lep.;Tortricidae) Egg Masses on Different Apple Cultivars. Asian Journal of Plant Sciences 6(6):982-987.
[23] Aydogdu, M. (2014) Parasitoid abundance of *Archips rosana* (Linnaeus, 1758) (Lepidoptera:Tortricidae) in Organic Cherry Orchards. North-Western Journal of Zoology 10(1): 42-47.
[24] Razmi, M., Karimpour, Y., Safaralizadeh, M.H. and Safavi, S.A. (2011) Parasitoid complex of cabbage large white butterfly *Pieris brassicae* (L.)(Lepidoptera, Pieridae) in Urmia with new records from Iran. Journal of Plant Protection Research 51(3): 248-251.
[25] Newman, I.C., Walker, J.T.S and Rogers, D.J. (2004) Mortality of the leafroller parasitoid *Dolichogenidea tasmanica* (Hym : Braconidae) exposed to orchard pesticide residues. New Zealand Plant Protection 57: 8-12.
[26] Alves, S.N., Tibúrcio, J.D. and Melo, A.L.D. (2011) Susceptibility of *Culex quinquefasciatus* larvae to different insecticides. Revista da Sociedade Brasileira de Medicina Tropical 44(4): 486-489.
[27] Sayyed, A.H., Haward, R., Herrero, S., Ferré, J. and Wright, D.J. (2000) Genetic and biochemical approach for characterization of resistance to *Bacillus thuringiensis* toxin Cry1Ac in a field population of the diamondback moth, *Plutella xylostella*. Applied and Environmental Microbiology 66(4): 1509-1516.
[28] Sayyed, A.H., Ahmad, M. and Saleem, M.A. (2008) Cross-resistance and genetics of resistance to indoxacarb in *Spodoptera litura* (Lepidoptera: Noctuidae). Journal of Economic Entomology 101(2): 472-479.
[29] Doganlar, O. (2008) Temperature-dependent development and degree-day model of European leaf roller, *Archips rosanus*. Journal of Plant Protection Research 48(1) 63-72.
[30] Hassan, S., Bigler, F., Bogenschütz, H., Boller, E., Brun, J, Calis, J., Coremans-Pelseneer, J,, Duso, C., Grove, A. and Heimbach, U. (1994) Results of the sixth joint pesticide testing programme of the IOBC/WPRS-Working Group «Pesticides and Beneficial Organisms». Entomophaga 39(1):107-119.
[31] Usmani, K.A. and Knowles, C.O. (2001) Toxicity of pyrethroids and effect of synergists to larval and adult *Helicoverpa zea*, *Spodoptera*

frugiperda, and *Agrotis ipsilon* (Lepidoptera: Noctuidae). Journal of Economic Entomology 94(4): 868-873.
[32] Zhang, Z.Y., Wang, D.L., Chi, Z.J., Liu, X.J. and Hong, X.Y. (2008) Acute toxicity of organophosphorus and pyrethroid insecticides to *Bombyx mori*. Journal of Economic Entomology 101(2): 360-364.
[33] Yee, W.L. and Alston, D.G. (2012) Behavioral responses, rate of mortality, and oviposition of western cherry fruit fly exposed to malathion, zeta-cypermethrin, and spinetoram. Journal of Pest Science 85(1): 141-151.
[34] Sallam, A.A., Volkmar, C., El-Wakeil, N.E. (2009) Effectiveness of different bio-rational insecticides applied on wheat plants to control cereal aphids. Journal of Plant Diseases and Protection 116(6): 283.
[35] Ahmad, M., Saleem, M.A. and Sayyed, A.H. (2009) Efficacy of insecticide mixtures against pyrethroid- and organophosphate-resistant populations of *Spodoptera litura* (Lepidoptera: Noctuidae). Pest Management Science 65(3): 266-274.
[36] Ahmad, M., Arif, M. and Attique, M. (1997) Pyrethroid resistance of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Pakistan. Bulletin of Entomological Research 87(4): 343-347.
[37] Sak, O. and Uçkan, F. (2009) Cypermethrin *Galeria melonella* L. (Lepidoptera: Pyralidae)'nın Pupalaşma ve ölüm oranlarına etkisi. Arı Dergisi 9(3): 88-96.
[38] Lugojja, F., Ogenga-Latigo, M. and Smit, N. (2001) Impact of defoliation on the agronomic performance of sweet potato in Uganda. African Crop Science Journal 9(1): 103-108.

Received: **21.11.2016**
Accepted: **09.02.2017**

CORRESPONDING AUTHOR

Mitat Aydogdu
Trakya University, Science Faculty, Biology Department, 22030 Edirne

e-mail: mitataydogdu@trakya.edu.tr