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LABORATORY EVALUATION OF THE EFFECT OF INSECTICIDES ON *CHRYSOPERLA CARNEA* (NEUROPTERA: CHRYSOPIDAE), *FORFICULA AURICULARIA* (DERMAPTERA: FORFICULIDAE), *ADALIA BIPUNCTATA* (COLEOPTERA: COCCINELLIDAE) AND *HARMONIA AXYRIDIS* (COLEOPTERA: COCCINELLIDAE)

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Abstract

The effect of 8 biopesticides and 23 synthetic pesticides on the eggs and larvae of *Chrysoperla carnea* (Stephens, 1836), adults of *Forficula auricularia* Linnaeus, 1758, larvae of *Adalia bipunctata* (Linnaeus, 1758) and adults of *Harmonia axyridis* (Pallas, 1773) have been evaluated under laboratory conditions. All of the tested biopesticides were harmless (causing mortality < 30%) or only slightly harmful (causing mortality 30–79%) to the tested natural enemies. The tested synthetic fungicides were harmless or slightly harmful, with the exception of myclobutanil, which was moderately harmful (causing mortality 90–99%) to *A. bipunctata* larvae. The tested synthetic insecticides were harmless or slightly harmful to *C. carnea* eggs and *F. auricularia* larvae. Various results were obtained for neonicotinoids ranging from the harmful effects (causing mortality > 99%) of thiacloprid on *A. bipunctata* larvae and *H. axyridis* adults to the harmless effects of acetamiprid on *F. auricularia* adults and *C. carnea* eggs. Thiacloprid, dodine and chlorpyrifos-methyl had effects on the behaviour of *F. auricularia* adults. The results confirmed the selectivity of the tested biopesticides that had no negative effects on the tested natural enemies.

Keywords: *Adalia bipunctata, Chrysoperla carnea, Forficula auricularia, Harmonia axyridis,* pesticides, natural enemies, bioassay

INTRODUCTION

Predators and parasitoids generally contribute to the reduction in pest populations in agricultural crops. However, their ability to prevent fully and reliably pest damage is limited. Hence, finding a balance between the use of insecticides and the survival of natural enemies is one of the main priorities of integrated pest management (IPM). As apple and pear growers shift to selective control strategies for major arthropod pests, there is often a noticeable increase in the densities of natural enemies in their orchards (Epstein *et al.*, 2000; Miliczky *et al.*, 2000). The reduction in pesticide use and long-term mating disruption for *Cydia pomonella* (Linnaeus, 1758) led to an increased abundance of natural enemies and reduction in *Cacopsylla pyricola* (Förster, 1848) outbreaks (Amarasekare *et al*., 2017). In contrast, the repeated application of less selective insecticides can promote biological imbalances in ecosystems and can lead to the loss of IPM function (Meissle *et al*., 2010). There is good evidence that insecticides, especially those used for *Cydia pomonella*, elevated *C. pyricola* to key pest status by suppressing its natural enemies (Westigard, 1973). Gallardo *et al*. (2016) documented that a stepwise increase in natural enemy risk values increased the total pest management costs by $46/ha and $44/ha in natural enemy unfavourable apple and pear orchards, respectively.

The utilization of insecticides with few negative impacts on beneficial arthropods is necessary. Knowledge of the selectivity of insecticides allows us to adjust the choice of the insecticides used based on the presence of beneficial species and their sensitive developmental stages at the time of application.

To protect habitats in agricultural and surrounding areas, testing the side effects of pesticides on non-target organisms is required for pesticide registration in the European Union (COUNCIL OF THE EUROPEAN COMMUNITIES, 1991; COUNCIL OF THE EUROPEAN COMMUNITIES, 1996). Selectivity towards the natural enemies of pests has already known for many registered insecticides. According to Commission Directive 96/12/EC (COUNCIL OF THE EUROPEAN COMMUNITIES, 1996), two standard pesticide-sensitive species, a parasitoid and a predatory mite (e.g., *Aphidius rhopalosiphi* DeStefani, 1902 and *Typhlodromus pyri* Scheuten, 1857), should be used in tests of insecticide side effects on arthropods. Only two additional species that are relevant to the intended use of the substance should also been tested. Therefore, knowledge about the selectivity of insecticides is usually incomplete.

Some insecticides are not entirely selective towards some beneficial species, e.g., acetamiprid was moderately harmful to *Chrysoperla carnea* larvae (Nasreen *et al*., 2005), and residues of acetamiprid and abamectin had moderate to high toxicity to hatched larvae of *Deraeocoris brevis* (Uhler, 1904) (Dong-Soon *et al*., 2006). The toxicity of insecticides can greatly differ for particular developmental stages of beneficial organisms, e.g., deltamethrin is harmless to the eggs of *C. carnea* (Giolo *et al*., 2009) and slightly harmful (Garzon *et al*., 2015) or moderately harmful (Maia *et al*., 2016) to the larvae of *C. carnea*. In addition, data for some insecticides obtained by different authors are inconsistent, e.g., abamectin has been assessed as harmless for *C. carnea* larvae by Bueno and Freitas (2004) but slightly harmful by Maia *et al*. (2016). Usually, only data about the side effects on a single developmental stage are available for particular species of natural enemies. Limited data are available about the side effects of biopesticides.

Chrysoperla carnea is present in a wide range of agricultural crops and is one of the most commonly used and commercially available natural enemies (Tauber *et al*., 2000). *Chrysoperla carnea* consumes a wide range of arthropods, such as aphids, scales, leafhoppers, whiteflies, psyllids, thrips, eggs and larvae of lepidopterans, and mites (Principi and Canard, 1984).

Forficula auricularia is the most common species from the order Dermaptera in the Czech Republic (Kočárek *et al*., 2005). Adults and larvae are important predators of many arthropods (e.g., Lepidoptera, Homoptera, Diptera). Earwigs also consume plant material and, for this reason, they can be sometimes classified as pest (Solomon *et al*., 2000).

Adalia bipunctata (Coleoptera: Coccinellidae) is commercially available and widely used to protect horticultural crops (Robledo Camacho *et al*., 2009). It consumes aphids and, together with other coccinellids, contributes to decreased population growth rates of aphids (Hodek and Honěk, 1996).

Harmonia axyridis (Coleoptera: Coccinellidae) was widely introduced as a biological control agent of aphids and coccids. On the other hand, this invasive alien ladybird in Europe has a negative effect on some native coccinelid species (Brown *et al*., 2008). The first occurrence of the species in the Czech Republic was documented in 2006 (Špryňar, 2008), and after a short period, it became a dominant species in some habitats (Honěk *et al*., 2016).

Harmonia axyridis and *Coccinella septempunctata* are the most important predators of aphids in orchards and hop gardens as well as *Cacopsylla* spp. (Hemiptera: Psyllidae) in pear orchards in the Czech Republic. Large populations of both species could reduce the number of insecticide treatments against these pests (1–2 per season). *Forficula auricularia* is a locally important predator of aphids and *Cacopsylla* spp.; nevertheless, its population density in orchards has decreased in the past 20–30 years, most likely due to new active substances registered for use in orchards. Added shelters on trees usually have no significant positive effect on *F. auricularia* populations. *Chrysoperla carnea* is common in all agricultural crops; nevertheless, the species population size is typically low, and it is only a complementary predator in the system (Holý and Stará, unpublished results). Populations of *A. bipunctata* have decreased since the 1980s (Honěk *et al*., 2016), and adults and larvae are rare in orchards and hop gardens.

The goal of the present study was to characterize the side effects of some pesticides and biopesticides used for pest control in orchards, hop gardens and field vegetables on selected natural enemy species and to prove the suitability of the tested pesticides for use in integrated pest management programmes. Pesticide selections were made on the basis of frequency of use in target crops in the Czech Republic and the knowledge of their side effects (whether they were not tested yet or if different published results contained contradictory information). We focused mainly on biopesticides and fungicides, which are less frequently tested. Twelve insecticides, 11 fungicides and 8 biopesticides registered in the Czech Republic were selected to test their effects on eggs and larvae of *Chrysoperla carnea* (Stephens, 1836) (Neuroptera: Chrysopidae), adults of *Forficula auricularia* (Linnaeus, 1758) (Dermaptera: Forficulidae), larvae of *Adalia bipunctata* (Linnaeus, 1758) (Coleoptera: Coccinellidae) and adults of *Harmonia axyridis* (Pallas, 1773) (Coleoptera: Coccinellidae).

MATERIALS AND METHODS

Insecticides

In total, 31 commercial formulations of insecticides, fungicides and biopesticides were tested against selected predators. The pesticides were diluted in distilled water in the concentrations recommended on the product labels, and Break-Thru SPU surfactant based on polyether-polymethylsiloxane-copolymer 80% and polyether 20% (Goldschmidt AG, Essen, Germany) in concentration 0.05% was added when appropriate (Tab. I). The *Quassia amara* L. extract was prepared 2–4 h before application by boiling *Quassia* wood chips (100 g) in 2 l of water for one hour (Psota *et al*., 2010). The insecticides tested against particular species were selected according to the available data about their selectivity against the tested species.

Insects

Chrysoperla carnea larvae originated from Koppert B. V. (Berkel en Rodenrijs, The Netherlands). The larvae (second instar) were mixed with buckwheat in 500 ml bottles. *Chrysoperla carnea* eggs originated from the laboratory colony of *C. carnea* maintained in the Institute of Entomology, Biology Centre CAS (České Budějovice, the Czech Republic). The eggs were on office paper.

Forficula auricularia adults were collected from paper belt traps placed on tree trunks in the experimental apple orchard of the Crop Research Institute, Prague, the Czech Republic. The earwigs were in groups of 20 and placed in 100 ml plastic cups. To minimize the stress of earwigs, pieces of grass were in cups as a shelter. The plastic cups with earwigs were transported in a styrofoam box to the laboratory. Earwigs were stored in a refrigerator at 5 °C for 24 h.

Adalia bipunctata larvae originated from Koppert B. V. (Berkel en Rodenrijs, The Netherlands*)*. The larvae (first instar) were with buckwheat in cotton bags. Eggs of *Sitotroga cerealella* (Olivier, 1789) were as food to avoid starvation during transport.

The *Harmonia axyridis* used in the test procedures originated from adults collected in an apple orchard in Bílé Podolí, the Czech Republic. Adults were collected in groups of 20 into 100 ml plastic cups. To minimize stress, pieces of grass were in the cups as a shelter. The plastic cups with *H. axyridis* were transported in a styrofoam box to the laboratory. Adults fed aphids and were stored at 20 °C for 24 h.

Test Procedures

The larvae of *C. carnea* were placed in groups of ten onto wet filter paper in 100 ml plastic cups with a perforated lid and incubated in the refrigerator at 5 °C for 6 h before the experiment. After incubation, the larvae were placed at 20 °C for 0.5 h, and only larvae with good fitness (moving in the cups) were selected for use in the experiment. The side effects of the insecticides against larvae of *C. carnea* were tested using a bioassay on filter paper (125 × 83 mm) treated with insecticides. The insecticide solutions were applied to filter paper by using an SG e1 hand sprayer (Biostep; Germany). The sprayed volume was 0.52 ± 0.1 ml per filter paper. The filter paper in the control group was treated with distilled water with Break-Thru 0.05%. After application, the filter papers were placed into the culture wares and stored in the laboratory for 1 h to dry. Larvae of *C. carnea* were introduced individually into a 24-well tissue culture ware (IWAKI & CO., Ltd., Tokyo, Japan), and the wells were closed with treated filter paper. The wares were turned upside down to enable contact of larvae with the filter paper. *Chrysoperla carnea* larvae (groups of ten per replication) were exposed to fresh insecticide residues for 24 h and incubated in a climatic chamber at 22 °C, 65% relative humidity and a photoperiod of 16 L:8 D h. Four replications were performed per treatment. After a period of 24 h, mortality was evaluated. The experiment was carried out without feeding the larvae.

A similar method as for larvae of *C. carnea* was used for larvae of *A. bipunctata*. The difference was in use of polypropylene (PP) autoclavable boxes with 50 places (File Cases for Micro Vials, Cat. No. 2-1535, NeoLab, Germany). The size of the filter paper was 140 × 70 mm, and the spray volume was 0.49 ± 0.1 ml per filter paper. The mortality was evaluated after 12 h.

The side effect of insecticides on *C. carnea* eggs were tested using a dip test. *Chrysoperla carnea* eggs (groups of ten per replication) laid on a piece of office paper were dipped for 5 s into a solution of pesticide in a 100 ml beaker. Four replications were performed per treatment. After the treatment, the eggs were placed on filter paper to dry. The dried eggs were placed into 250 ml plastic cups with a perforated lid and wet filter paper at the bottom and incubated in a climatic chamber at 22 °C, 65% relative humidity and a photoperiod of 16 L:8 D h. Evaluations of egg mortality were performed after hatching all the eggs, i.e., after a week since the first eggs hatched.

I: *The trade names, active ingredients, companies and concentrations of pesticides used in the study*

Pesticide type	Trade name	Active ingredient (a.i.)	A.I./l or kg	Concentration [%]	Company
Insecticides	Actara 25 WG	thiamethoxam	0.25	0.008	Syngenta Crop Protection AG
	Calypso 480 SC	thiacloprid	0.48	0.025	Bayer CropScience AG
	Decis flow 2.5	deltametrin	0.025	0.04	Bayer CropScience AG
	Insegar 25 WG	fenoxycarb	0.25	0.06	Syngenta Crop Protection AG
	Integro	methoxyfenozide	0.24	0.05	Dow AgroSciences s. r. o.
	Karate with Zeon tech. 5 CS	lambda-cyhalotrin	0.05	0.025	Syngenta Limited
	Mospilan 20 SP	acetamiprid	0.2	0.025	Nisso Chemical Europe GmbH.
	Reldan 22	chlorpyrifos-metyl	0.225	0	Dow AgroSciences s. r. o.
	Spintor	spinosad	0.24	0.08	Dow AgroSciences s. r. o.
	Steward	indoxacarb	0.3	0.017	Du Pont CZ, s.r.o.
	Vertimec 1,8 EC	abamectin	0.018	0.1	Syngenta Crop Protection AG
Fungicides				0	
	Antre 70 WG	propineb	0.7	0.225	Bayer CropScience AG
	Domark 10 EC	tetraconazole	0.1	0.04	Isagro s. r. l.
	Fandango 200 EC	fluoxastrobin, prothioconazole	0.1; 0.1	0.3125	Bayer CropScience AG
	Chorus 75 WG	cyprodinil	0.75	0.02	Syngenta Crop Protection AG
	Infinito	fluopicolide, propamocarb-hydrochloride	0.0625; 0.625	0.4	Bayer CropScience AG
	Revus 250 SC	mandipropamid	0.25	0.15	Syngenta Crop Protection AG
	Signum	boscalid, pyraclostrobin	0.267; 0.067	0.075	BASF AG
	Switch	cypronidil, fludioxonil	0.375; 0.25	0.1	Syngenta Crop Protection AG
	Syllit 65 WP	dodine	0.65	0.17	Agrobio
	Talent	myclobutanil	0.2	0.07	Dow AgroSciences s. r. o.
	Thiram granuflo	thiram	0.8	0.3	Taminco N.V.
Biopesticides					0
	Alginure	seaweed extract	0.324	0.5	Biocont Laboratory
	Aqua-vitrin	water glass	0.285	0.002	Biocont Laboratory
	Cocana*	coconut soap	0.27	0.7	Biocont Laboratory
	Ekol*	rape seed oil	0.9	2	Proxim, s.r.o.
	Myco-Sin	sulfuric acid clay ($Al_2(SO_4)_3$), deactivated yeast components, extract from horsetail	0.74; 0.14; 0.01	1	Biocont Laboratory
	Prev-B2*	orange oil, fatty alcohol ethoxylate	-	0.3	Biocont Laboratory
	Quassia amara	quassin, neoquassin	-	0.45	Biocont Laboratory
	Rock Effect*	*Pongamia pinnata* oil	0.868	1	Agro CS
	Vitisan	kalium hydrogencarbonate	0.995	0.5	Biofa AG

Products marked with * were used without Break-Thru addition (concentration 0.05%)

A topical application bioassay was used to test the side effect of insecticides on *F. auricularia* adults. Before the experiment, earwigs were sorted into groups of 10 into 100 ml plastic cups with perforated lids and dry filter paper at the bottom. Pieces of apples were provided as food for the duration of the experiment. The earwigs were inhibited by CO_2, and a 1-µl volume of pesticide dissolved in acetone was applied topically to each earwig with a Multipette Plus dispenser (Eppendorf AG, Hamburg, Germany). Pure acetone was used in the control variant. No Break-Thru surfactant was used in this experiment. The treated earwigs in plastic cups were incubated in a climatic chamber at 22 °C, 65% relative humidity and a photoperiod of 16 L:8 D h. After a period of 48 h, the mortality of earwigs was evaluated.

Harmonia axyridis adults were sorted into groups of 10 into 100 ml plastic cups (90 × 50 mm) with perforated lids and dry filter paper at the bottom. Each cup was treated with 0.32 ± 0.1 ml of solution sprayed as a mist over the beetle (Smith and Kirschik, 2000) by using an SG e1 hand sprayer (Biostep, Germany). The treated coccinelids in the plastic cups were incubated in a climatic chamber at 22 °C, 65% relative humidity and a photoperiod of 16 L:8 D h. After a period of 24 and 48 h, the mortality of adults was evaluated. The experiment was carried out without feeding adults. Only insects that had good fitness were used for the bioassays.

Statistical Analysis

The data of *C. carnea* eggs, *F. auricularia* adults, *A. bipunctata* larvae and *H. axyridis* adults, presented as the mean of mortality ± SD, were analysed using one-way analysis of variance (ANOVA) after testing their normality with a Shapiro-Wilk test using the XLSTAT 2015 statistical software package (Addinsoft Inc., New York, USA). The means were separated using Tukey's HSD analysis. The data for the *C. carnea* larval mortality did not follow a normal distribution and were analysed using the nonparametric Kruskal-Wallis test following the multiple pairwise comparisons using the Steel-Dwass-Critchlow-Fligner procedure. The mortality data corrected using Abbott (1925) are presented in tables. According to the IOBC laboratory scale (Hassan, 1994) and the mortalities corrected by the control mortality (Abbott, 1925), the pesticides were classified into four toxicity categories:

1. harmless (< 30%);
2. slightly harmful (30–79%);
3. moderately harmful (80–99%); and
4. harmful (> 99%).

RESULTS

Lethal Effects of Insecticides on *Chrysoperla carnea* Mortality

According to the results of ANOVA model, differences in the mortality of *C. carnea* eggs were found after 24 h ($F = 4.476$, $df = 6, 21$, $p = 0.005$) of exposure to residues of insecticides (Tab. II). The mortality in the control group reached 15.5% of *C. carnea* eggs. Most of the tested insecticides were harmless to *C. carnea* eggs, causing corrected mortality from 0% (chlorpyrifos-

II: *Pesticide side effects on Chrysoperla carnea eggs and placement into IOBC side-effect classes. Values with different small letters are significantly different.*

Pesticide	N	Emerged larvae	*Mortality of eggs %	sd	IOBC class
Reldan 22	40	36	0^{a}	0	1
Decis Flow 2.5 EC	44	41	0^{a}	0	1
Steward	56	36	18.8^{ab}	21.5	1
Mospilan 20 SP	47	31	21.4^{ab}	7.00	1
Spintor	52	33	24.1^{ab}	17.9	1
Integro	42	23	32.0^{b}	22.1	2

*corrected mortality by Abbott
sd = standard deviation

III: *Pesticide side effects on Chrysoperla carnea larvae and placement into IOBC side-effect classes. Values with different small letters are significantly different.*

Pesticide	N	Dead larvae during 1st moulting	*Mortality %	sd	IOBC class
Alginure	40	0	0^{a}	0	1
Aqua-vitrin	40	1	2.5^{a}	5.0	1
Myco-Sin	40	0	0^{a}	0	1
Prev-B2	40	0	0^{a}	0	1
Rock effect	40	0	0^{a}	0	1
Vitisan	40	0	0^{a}	0	1

*corrected mortality by Abbott
sd = standard deviation

methyl and deltamethrin) to 24.1% (spinosad) 24 h after application (Tab. II). Methoxyfenozide was slightly harmful, causing 32.0% corrected mortality of *C. carnea* eggs after 24 h.

According to results of the Kruskal-Wallis test, no significant differences in the mortality of *C. carnea* larvae were found after 24 h (KW-$H_{6,4}$ = 6, α = 0.05, p = 0.423) of exposure to dry biopesticide residues (Tab. III). No mortality was recorded in the control variant. All of the tested biopesticides were harmless to *C. carnea* larvae. Except for water glass, which causes a 2.50% mortality of *C. carnea* larvae, all of the tested biopesticides caused no mortality of *C. carnea* larvae (Tab. III).

Side Effects of Insecticides on *Forficula auricularia* Mortality

The ANOVA model showed differences in the mortality of *F. auricularia* adults after 48 h (F = 148.438, df = 15, 49, p < 0.0001) from the application of synthetic pesticides and biopesticides (Tab. IV). The mortality in the control group reached 1.1%. All of the tested synthetic fungicides and biopesticides were harmless to *F. auricularia* adults, causing mortality from 0% to 1.38% (seaweed extract) 48 h after application (Tab. IV). Chlorpyrifos-methyl was slightly harmful, causing 57.5% mortality of *F. auricularia* adults after 48 h. The rest of the tested synthetic insecticides were harmless, causing no mortality of *F. auricularia* adults (Tab. IV). Chlorpyrifos-methyl caused 57.5% mortality, but the rest of the surviving individuals showed dramatically changed behaviour with reduced mobility. Similar behavioural changes were observed in dodine and thiacloprid variants.

Side Effects of Insecticides on *Adalia bipunctata* Mortality

The ANOVA results showed large differences in the sensitivity of *A. bipunctata* larvae to the tested synthetic pesticides and biopesticides. The mortality of *A. bipunctata* larvae differed significantly after 24 h of application of the tested pesticides (F = 29.125, df = 14, 30, p < 0.0001) (Tab. V). The mortality in the control variant reached 16.7%. Most of the tested biopesticides were harmless to *Adalia bipunctata* larvae, causing corrected mortality values from 0% (hydrogencarbonate) to 16.0% (sulfuric acid clay ($Al_2(SO_4)_3$), deactivated yeast components, extract from horsetail). The toxic effect of the tested synthetic fungicides varied from harmless to moderately harmful to *A. bipunctata* larvae, causing mortality from 20.0% (boscalid + pyraclostrobin in Signum) to 92.0% (myclobutanil). However, most of the tested synthetic fungicides were slightly harmful to *A. bipunctata* larvae. The tested neonicotinoids acetamiprid and thiacloprid were moderately harmful and harmful, respectively, to *A. bipunctata* larvae, causing mortality rates of 88.0% and 100% (Tab. V).

Side Effects of Insecticides on *Harmonia axyridis* Mortality

The ANOVA results showed significant differences in the mortality of *H. axyridis* adults 24 h after the application of tested synthetic pesticides and biopesticides (F = 271.251, df = 20, 63, p < 0.0001) (Tab. VI). No mortality was observed in the control variant. All the tested biopesticides and synthetic fungicides were harmless to *H. axyridis* adults, causing mortality from 0% to 7.50% (coconut soap and propineb). Large differences in toxicity

IV: *Pesticide side effects on Forficula auricularia adults and placement into IOBC side-effect classes. Values with different small letters are significantly different.*

Pesticide	N	Dead adults	*Mortality of adults %	sd	IOBC class
Alginure	72	1	1.38a	3.67	1
Vitisan	80	1	1.25a	3.31	1
Calypso 480 SC	41	0	0a	0	1**
Insegar 25 WG	42	0	0a	0	1
Mospilan 20 SP	30	0	0a	0	1
Mycosin	78	0	0a	0	1
Quassia amara	30	0	0a	0	1
Rock Effect	39	0	0a	0	1
Syllit 65 WP	40	0	0a	0	1**
Thiram granuflo	30	0	0a	0	1
Vertimec 1.8 EC	41	0	0a	0	1
Reldan 22	40	23	57.5b	4.33	2**

*corrected mortality by Abbott
** effect on behaviour
sd = standard deviation

V: *Pesticide side effects on Adalia bipunctata larvae and placement into IOBC side-effect classes. Values with different small letters are significantly different.*

Pesticide	N	Dead larvae during 1st molting	*Mortality %	sd	IOBC class
Vitisan	30	4	0[a]	6.93	1
Alginure	30	7	7.96[ab]	6.93	1
Prev-B2	30	8	12.0[ab]	6.93	1
Myco-Sin	30	9	16.0[ab]	20.8	1
Signum	30	10	20.0[ab]	18.3	1
Cocana	30	13	32.0[bc]	13.9	2
Chorus 75 WG	30	19	56.0[cd]	6.93	2
Fandango 200 EC	30	20	60.0[cde]	18.3	2
Infinito	30	21	64.0[cde]	12.0	2
Revus 250 SC	30	21	64.0[cde]	12.0	2
Switch	30	23	72.0[def]	6.93	2
Mospilan 20 SP	30	27	88.0[def]	0	3
Talent	30	28	92.0[ef]	6.93	3
Calypso 480 SC	30	30	100[f]	0	4

*corrected mortality by Abbott
sd = standard deviation

VI: *Pesticide side effects on Harmonia axyridis adults and placement into IOBC side-effect classes. Values with different small letters are significantly different.*

Pesticide	N	dead adults	*mortality %	sd	IOBC class
Alginure	40	0	0a	0	1
Chorus 75 WG	40	0	0a	0	1
Ekol	40	0	0a	0	1
Infinito	40	0	0a	0	1
Myco-Sin	40	0	0a	0	1
Prev-B2	40	0	0a	0	1
Revus 250 SC	40	0	0a	0	1
Swich	40	0	0a	0	1
Talent	40	0	0a	0	1
Vitisan	40	0	0a	0	1
Fandango 200 EC	40	1	2.50a	4.33	1
Signum	40	1	2.50a	4.33	1
Domark 10 EC	40	1	2.50a	4.33	1
Cocana	40	3	7.50a	8.29	1
Antre 70 WG	40	3	7.50a	8.29	1
Actara 25 WG	40	12	30.0b	7.07	2
Calypso 480 SC	40	40	100c	0	4
Karate with Zeon technol.	40	40	100c	0	4
Mospilan 20 SP	40	40	100c	0	4

*corrected mortality by Abbott
sd = standard deviation

were obtained for the tested neonicotinoids. Thiamethoxam was slightly harmful, with 30.0% mortality of *H. axyridis*, while thiacloprid and acetamiprid were harmful, causing 100% mortality. The organophosphate chlorpyrifos-methyl was also harmful, causing 100% mortality of *H. axyridis* adults (Tab. VI).

DISCUSSION

The success of IPM and organic regimes is closely connected with the survival of beneficial organisms in the environment. Beneficials are more important in organic regimes due to the low number of available insecticides. More knowledge is known about the side effects of synthetic insecticides than those of biopesticides and fungicides. Nevertheless, biopesticides could also have negative effects on beneficials. Hence, complete information on the selectivity and possible side effects of pesticides, especially newly registered ones, is necessary to maintain an IPM and organic regime functional in particular crops.

Among the insecticides tested against the four species *C. carnea*, *F. auricularia*, *A. bipunctata* and *H. axyridis*, the neonicotinoids thiacloprid and acetamiprid had diverse effects ranging from harmless against *C. carnea* larvae to harmful against *H. axyridis* adults. There was great diversity in the toxicity of acetamiprid according to species, ranging from the harmless effect on *Neoseiulus cucumeris* to the harmful effect on *Aphidius colemani*, was also found in our previous work (Stará *et al.*, 2014). Thiacloprid does not kill *F. auricularia* adults, but it does have a negative effect on their mobility. Negative behavioural changes were observed also in the adults who survived the chlorpyrifos-methyl variant. Thiamethoxam was the least toxic neonicotinoid from the three neonicotinoids tested and was only slightly harmful to the adults of *H. axyridis*. Similar results were obtained by Galvan *et al.* (2006).

Most of the tested fungicides were harmless to *H. axyridis* adults and *F. auricularia* adults, except dodine, which caused no mortality of *F. auricularia* but same as thiacloprid had negative effect on behavioural changes of all adults. In all cases, larvae of *A. bipunctata* were more sensitive to tested fungicides than *H. axyridis* adults. Most of the tested fungicides were slightly harmful to *A. bipunctata*. Large differences in the toxicities of substances against the developmental stages of tested Coccinellidae were found for myclobutanil, which was harmless to *H. axyridis* larvae but moderately harmful to *A. bipunctata* larvae. Regarding the wide range of mortality (30–79%) in toxicity category 2, which is slightly harmful according to the IOBC laboratory scale, the use of fungicides in the period of presence of Coccinellidae larvae in orchards should be carefully considered.

All of the tested biopesticides were harmless to *C. carnea* larvae, *F. auricularia* adults and *H. axyridis* adults. Again, *A. bipunctata* larvae proved to be less tolerant than *H. axyridis* adults, e.g., in the case of Cocana (coconut soap) and Myco-sin (sulfuric acid clay ($Al_2(SO_4)_3$), deactivated yeast components, extract from horsetail).

In general, the 1st instar larvae of *A. bipunctata* were the most sensitive to the tested pesticides, which corresponds with Olszak (1999), while *F. auricularia* adults and both tested stages of *C. carnea* were tolerant. According to Medina *et al.* (2008), *C. carnea* is considered relatively tolerant to pesticides, but the larvae are more susceptible than adults (Giolo *et al.*, 2009). In our experiment, eggs of *C. carnea* were tolerant to all of the tested insecticides, including acetamiprid, which was slightly harmful and harmful to *A. bipunctata* and *H. axyridis*, respectively. Similarly, *C. carnea* eggs were tolerant to deltamethrin, while *lambda*-cyhalothrin, which is from the same class II of pyrethroids, was harmful to *H. axyridis* adults. Spinosad was harmless to *C. carnea* eggs in our experiment but was slightly harmful to *A. aphidimyza* and *N. cucumeris* and harmful to *A. colemani* (Stará *et al.*, 2011).

CONCLUSION

The tested biopesticides had no negative effects on *Chrysoperla carnea*, *Forficula auricularia*, *Adalia bipunctata* and *Harmonia axyridis*. The synthetic fungicides were harmless or only slightly harmful to the tested natural enemies. *Forficula auricularia* adults and both tested stages of *C. carnea* were tolerant to the tested pesticides. In general, *A. bipunctata* larvae were more sensitive to the tested pesticides than *H. axyridis* adults. Both *A. bipunctata* larvae and *H. axyridis* adults were sensitive to the tested neonicotinoids.

From a practical point of view, only the pesticides that were classified as harmless or slightly harmful can be considered suitable for use in integrated or organic regimes. In fact, a mortality of beneficial species higher than 80% is too high to enable the beneficial species to alter the dynamics of pest populations. Hence, moderately harmful pesticides do not differ from the pesticides that were classified as harmful regarding their real impact on beneficial species. Our results bring other information to the mosaic of knowledge of the side effects of pesticides and could be used for improving IPM and organic regimes in different crops.

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