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Toxicity of greenhouse pesticides to multicolored Asian lady beetles, *Harmonia axyridis* (Coleoptera: Coccinellidae)

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Abstract

A variety of insecticides, acaricides, and fungicides is used in greenhouses to protect plants. As predacious ladybirds are also used to control aphids, it is important to understand the effects of such pesticides on these beneficial insects. We examined the susceptibility to all developmental stages of the multicolored Asian ladybird beetle, *Harmonia axyridis* (Pallas), to 12 agents: four insecticides, four acaricides and four fungicides. Etofenprox and acetamiprid were highly toxic to most developmental stages, and also in the adult at 200 mg [AI]/L and 40 mg [AI]/L (recommended dosages for aphid control). Thiamethoxam caused knockdown of larvae, pupae, and adults; however, most recovered within 24 h. Imidacloprid, applied at 50 mg [AI]/L, produced LC_{50} values of 30.3 and 190.2 mg [AI]/L for 3rd and 4th instars, respectively. Abamectin was highly toxic to eggs, larvae, pupae, and adult ladybirds at rates under 18.4 mg [AI]/L. However, recommended doses of other acaricides were very safe to all stages except for the egg stage of *H. axyridis*. The fungicide pyrazophos was highly toxic to eggs and larvae at a rate much lower than the recommended dose, while other tested fungicides were not toxic to any of the ladybird stages at rates over 1000 mg [AI]/L. Generally, the 1st and 2nd instars of *H. axyridis* were very sensitive to most of the tested insecticides and acaricides. Fungicides, on the other hand, were generally harmless to *H. axyridis*.

Keywords: *Harmonia axyridis*; IPM; Insecticides; Acaricides; Fungicides

1. Introduction

The multicolored Asian ladybird beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), is common to a wide range of natural and agricultural habitats with worldwide distribution. It is a generalist predator that feeds primarily on several aphid species (Seo and Youn, 2000) and has been recognized for its potential contribution to the integrated management of various crop aphids. These include the green peach aphid, the cotton aphid, the turnip aphid, and the corn leaf aphid.

Insect pest management in greenhouses relies on both natural enemies and insecticides, as is typical of many agroecosystems. Natural enemies control a number of pests, while others are dependent on chemical control. In a natural community, aphid predators, in particular Coccinellidae, Syrphidae, and Cecidomyiidae, are important and can cause severe aphid mortality. Natural enemies of aphids can reduce the rate of population increase, occasionally dramatically, and the use of ladybirds in biological control of aphids can be very successful (Seo and Youn, 2000, 2002). Theiling and Croft (1988) showed that coccinellid susceptibility to insecticides varies with the species and the type of pesticide and suggested that the sensitivity of coccinellids to insecticides was often slightly lower than that of pests.

Within most crop ecosystems, some pests have no effective biological control agents (Castane et al., 1996). However, the frequent use of insecticides to manage these pests may destroy natural enemies and encourage pest resurgence or a secondary pest outbreak. A key principle of integrated pest management (IPM) is to maximize pest control from natural mortality factors such as predators and parasitoids. These are supplemented where necessary with pesticides, which should be used in a way that

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minimizes the disruption of biological control agents. It is important that chemical agents, spray thresholds, and application schedules are compatible with natural enemies. Several studies have aimed at standardizing methods for evaluating pesticide effects on beneficial insects (e.g., Franz, 1975). In this study, we examined the susceptibility of different stages of *H. axyridis* to 12 agents: four insecticides, four acaricides, and four fungicides.

2. Materials and methods

2.1. Insects

Harmonia axyridis were reared on the cotton aphid, *Aphis gossypii*, in the laboratory and overwintering adult *H. axyridis* were collected at aggregation sites in the Chungnam and Daejon areas from mid November to late December in 1997 and 1998. These insects were placed in 15 cm diameter plastic petri dishes and reared at 15 °C constant temperature under dark conditions. They were fed on ground chicken liver with a 10% sucrose solution (v/v), which was renewed every three days. Reproductive adult females were removed from the petri dishes, reared with the cotton aphid in the laboratory, and allowed to lay eggs. First generation progeny were also reared on *A. gossypii* in plastic containers ($30 \times 30 \times 40$ cm) under 25 ± 1 °C, 50–60% RH, and 16:8 (L:D) photoperiod conditions.

2.2. Pesticides

Each of the four insecticides (acetamiprid, etofenprox, imidacloprid, and thiamethoxam), acaricides (abamectin, chlorfenapyr, fenazaquin, and tebufenpyrad) and fungicides (dichofluanid, difenoconazole, procymidone, and pyrazophos) were tested. These compounds were selected on the basis of their current and potential use for the management of key greenhouse insect, mite, and disease pests affecting pepper, tomato, cucumber, and other vegetables. They were also selected because they represented a variety of chemical families. Details of such elements as target pests, rates and formulations of the pesticides are listed in Table 1.

2.3. Bioassay

A 100-ml stock solution (each pesticide was diluted with 80% (v/v) acetone in a water solution) was prepared for each pesticide with concentrations reflecting recommended field rates by producers (Table 1). For LC_{50} values, concentrations were progressively altered until 50% mortality was achieved. All instars and the adult stage were treated topically with 1 μl on the ventral abdomen with a microapplicator (Burkard, England). Egg batches and pupae were dipped in the chemicals for 10 s, followed by cleaning with filter paper (Whatman No. 2, Kent, England), taking care to absorb and dry the smeared solution because they were attached and fixed on the substances. Controls were tested with 80% acetone using the same methods. Ladybirds were held at 25 ± 1 °C, 50–60% RH, and 16:8 (L:D) until egg hatch or adulthood. For equalizations between repetitions, eggs were used 24 h after being laid. First and second instars were used 24 h after laying and molting due to the short period before the next stage. Third and fourth instar, pupae, and adults were treated for 48 h after molting, pupating, and emergence, respectively. Before

Table 1
Chemicals tested for toxicity to the multicolored Asian ladybird beetle, *Harmonia axyridis*

Chemistry	Common names	Families	Trade name	Formulation[a]	Recommended concentrations (mg[AI]/L)	Main targets	Manufacturing company
Insecticides	Acetamiprid	Chloronicotinyl	Mospilan	WP	40	Aphids	Kyungnong
	Etofenprox	Non-ester pyrethroid	Sebero	EC	200	Whitefly, Beet army worm	Kyungnong
	Imidacloprid	Imidazolidine	Confidor	WP	50	Aphids, Thrips, etc	DongbuHannong
	Thiamethoxam	Thiamicotinyl	Actara	WG	50	Aphids, Thrips, etc	Novartis
Acaricides	Abamectin	Avermectin	Allstar	EC	9	Mites, Thrips, Moth larvae, etc	Kyungnong
	Chlorfenapyr	Pyrroles	Rempage	FC	100	Mites, Thrips, Moth larvae, etc	Kyungnong
	Fenazaquin	Quinazoline	Boramae	EC	67	Mites	Kyungnong
	Tebufenpyrad	Pyrazole	Pyranica	EC	50	Mites	Novartis
Fungicides	Dichofluanid	*N*-trihalomethylthio	Euparen	WP	1000	Gray mold	DongbuHannong
	Difenoconazole	Triazole	Purgen	EC	50	Black rot	Novartis
	Procymidone	Dicarboximide	Sumilex	WP	500	Gray mold	Aventis
	Pyrazophos	Organophospate ester	Afugan	EC	300	Powdery mildew	Aventis

[a] EC, emulsifiable concentrate; WP, wettable powder; WG, wettable granule; FC, flowable concentrate.

the treatments, larvae and adult ladybirds were immobilized in a refrigerator at −4 °C for 10–30 s, depending on the stage of development. Five insects (or egg batches) were tested per replicate, three replicates were performed for each test stage and the insects were tested on different days with different generations. There were different test stages for LC_{50} values for each chemical. After treatment, the insects were maintained with the cotton aphid in plastic petri dishes under the same conditions. Mortality was determined 48 h after treatment for the mobile stages, the criterion for death being the failure of the ladybird to move its legs when stimulated with a fine brush. In the case of eggs and pupae, one week was allowed to enable them to either hatch or reach adulthood.

2.4. Statistical analysis

Survival rates of the ladybirds tested with pesticides were analyzed by analysis of variance with a completely randomized design in SAS (SAS Institute, 1991). Data for LC_{50} values were analyzed by probit analysis (1987 version) (Finney, 1971).

3. Results

3.1. Survival rate bioassay

Eggs of *H. axyridis* exposed to acetamiprid, imidacloprid, and abamectin produced no larvae, while those exposed to pyrazophos, etofenprox, fenazaquin, tebufenpyrad, and chlorfenapyr had survival rates of 11.4–54.1% (Table 2). Survival rates for tebufenpyrad and chlorfenapyr were uneven, ranging from 28–82% and 5–95%, respectively. Dichofluanid, difenoconazole, and procymidone had no effect on hatching. When 1st and 2nd instars were exposed to acetamiprid, etofenprox, imidacloprid, and abamectin, the survival rate was zero. However, 40–60% of 1st and 100% of 2nd instars survived treatment with thiamethoxam. This chemical caused knockdown of instars and adults, but most recovered within 24 h of application. When 3rd and 4th instars were exposed to acetamiprid, etofenprox, and pyrazophos, the survival rate was zero, while imidacloprid, abamectin, and thiamethoxam were only slightly harmful. Pupae exposed to acetamiprid and abamectin produced no adults, but were only slightly affected by pyrazophos. Acetamiprid was most harmful to adult *H. axyridis*, followed by abamectin, thiamethoxam, etofenprox, and imidacloprid (Table 2). Three of the acaricides and fungicides were safe to larval, pupal, and adult stages. Pyrazophos was toxic to larvae and, to lesser extent, the pupae of *H. axyridis*.

3.2. LC_{50} bioassay

Relative toxicities of the 12 pesticides to stages of *H. axyridis* are shown in Tables 3 and 4. The LC_{50} values indicate that abamectin (<0.09 mg [AI]/L) was the most toxic compound to eggs and 1st and 2nd instars, followed by acetamiprid (<4.00 mg [AI]/L), imidacloprid (<8.79 mg [AI]/L), and etofenprox (<8.80 mg

Table 2
Mean survival rate (±SEM) for developmental stages and adult multicolored Asian ladybird beetle, *Harmonia axyridis*, topically treated or dipped in insecticides, acaricides, and fungicides at rates requested for use in greenhouses (mg[AI]/L)

	Common names	Developmental stages of *Harmonia axyridis*						
		Eggs	1st larvae	2nd larvae	3rd larvae	4th larvae	Pupae	Adults
Insecticides	Acetamiprid	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0d	0.0 ± 0.0c	0.0 ± 0.0c	6.7 ± 11.5d
	Etofenprox	26.5 ± 13.3bc	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0d	0.0 ± 0.0c	100.0 ± 0.0a	93.3 ± 11.5a
	Imidacloprid	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0c	26.7 ± 30.6c	90.0 ± 10.0a	100.0 ± 0.0a	100.0 ± 0.0a
	Thiamethoxam	92.5 ± 3.3a	46.7 ± 11.5b	100.0 ± 0.0a	86.7 ± 11.5a	93.3 ± 11.5a	100.0 ± 0.0a	86.7 ± 11.5ab
Acaricides	Abamectin	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0c	60.0 ± 34.6b	70.0 ± 10.0b	0.0 ± 0.0c	33.3 ± 30.6c
	Chlorfenapyr	54.1 ± 45.4b	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	73.3 ± 11.5b
	Fenazaquin	27.6 ± 15.2bc	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
	Tebufenpyrad	53.4 ± 27.2b	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
Fungicides	Dichofluanid	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
	Difenoconazole	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
	Procymidone	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
	Pyrazophos	11.4 ± 6.7c	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0d	0.0 ± 0.0c	53.3 ± 41.6b	20.0 ± 0.0cd
Control	80% of acetone	97.1 ± 2.1a	98.3 ± 0.8a	98.6 ± 1.1b	99.8 ± 0.8a	100.0 ± 0.0a	99.5 ± 0.5a	99.5 ± 0.5a
	C.V. value	40.0	5.6	0.8	19.8	7.1	14.2	12.8

Means followed by different letters are significantly different within a column for each chemical ($P < 0.05$, Duncan multiple test [SAS Institute, 1989]).

Table 3
Toxicity of insecticides, acaricides, and fungicides to eggs, 1st, 2nd, and 3rd instars of *Harmonia axyridis*

		Eggs			First instars			Second instars			Third instars		
		No. tested	LC_{50}		No. tested	LC_{50}		No. tested	LC_{50}		No. tested	LC_{50}	
			(mg AI/L)	95% FL		(mg AI/L)	95% FL		(mg AI/L)	95% FL		(mg AI/L)	95% FL
Insecticides	Acetamiprid	239	<4.00	—	75	<4.00	—	75	<4.00	—	75	<4.00	—
	Etofenprox	483	<8.80	—	75	<8.80	—	75	<8.80	—	75	72.71	45.1–121.4
	Imidacloprid	342	<8.79	—	75	<8.79	—	75	<8.79	—	105	30.3	16.7–41.7
	Thiamethoxam	329	382.31	113.1–1294.5	90	37.01	18.6–57.4	75	81.09	71.8– 89.6	105	124.03	100.1–145.5
Acaricides	Abamectin	284	<0.09	—	75	<0.09	—	75	<0.09	—	75	<0.09	—
	Chlorfenapyr	291	114.59	84.7–182.1	90	383.29	323.3–44.6	75	>2000.00	—	75	>2000.00	—
	Fenazaquin	497	16.44	6.4–42.2	150	123.96	111.3–46.5	105	488.03	391.9– 591.6	150	>5000.00	—
	Tebufenpyrad	525	33.52	14.7–76.1	90	251.54	202.1–283.8	180	346.03	325.9– 368.9	105	1190.98	842.2–3388.1
Fungicides	Dichofluanid	423	>1000.00	—	75	>1000.00	—	75	>1000.00	—	75	>1000.00	—
	Difenoconazole	236	>1000.00	—	75	>1000.00	—	75	>1000.00	—	75	>1000.00	—
	Procymidone	323	>1000.00	—	75	>1000.00	—	75	>1000.00	—	75	>1000.00	—
	Pyrazophos	402	50.94	39.1–62.1	75	<10.00	—	75	<10.00	—	75	26.65	22.1–31.0

Data for LC_{50} values were analyzed by probit analysis program (1987 version).

Table 4
Toxicity of insecticides, acaricides, and fungicides to 4th, pupae, and adults of *Harmonia axyridis*

		Fourth instars			Pupae			Adults		
		No. tested	LC_{50} (mg AI per L)	95% FL	No. tested	LC_{50} (mg AI per L)	95% FL	No. tested	LC_{50} (mg AI per L)	95% FL
Insecticides	Acetamiprid	165	5.80	0.7–29.1	75	<5.00	—	180	16.69	9.0–23.4
	Etofenprox	180	99.20	77.3–129.6	180	790.48	650.8–988.2	151	263.36	241.3– 312.4
	Imidacloprid	182	190.20	114.0–323.9	160	>1000.00	—	186	364.07	277.9–517.1
	Thiamethoxam	165	249.23	172.0–236.6	160	>2500.00	—	180	150.46	35.3–238.3
Acaricides	Abamectin	183	18.40	12.2–24.6	75	<0.09	—	155	4.88	2.5–13.6
	Chlorfenapyr	180	>2000.00	—	160	>2000.00	—	150	207.19	106.7–259.3
	Fenazaquin	240	>5000.00	—	180	1375.62	1265.2– 1511.3	120	>5000.00	—
	Tebufenpyrad	180	1190.98	842.2–3388.1	160	>2000.00	—	120	>3000.00	—
Fungicides	Dichofluanid	75	>1000.00	—	75	>1000.00	—	75	>1000.00	—
	Difenoconazole	75	>1000.00	—	75	>1000.00	—	75	>1000.00	—
	Procymidone	75	>1000.00	—	75	>1000.00	—	75	>1000.00	—
	Pyrazophos	135	39.19	31.2 – 52.3	150	460.62	351.7–883.6	145	221.4	186.4–262.8

Data for LC_{50} values were analyzed by probit analysis program (1987 version).

[AI]/L) (Table 3). Fenazaquin, tebufenpyrad, and pyrazophos were more specifically toxic to ladybird eggs. However, LC_{50} values of thiamethoxam (382.31 mg [AI]/L) and chlorfenapyr (114.59 mg [AI]/L) were much higher than the recommended concentrations, at 50 and 100 mg [AI]/L, respectively. The three fungicides (dichofluanid, difenoconazole, and procymidone) were non-toxic to eggs, larvae, pupae, and adults.

In the case of 1st instars of the ladybird, LC_{50} values of thiamethoxam were higher than the recommended concentrations. With the 3rd instar ladybirds in Table 3, LC_{50} values of abamectin, acetamiprid, etofenprox, imidacloprid, and pyrazophos were maintained at harmful levels. Acetamiprid, etofenprox, and pyrazophos could kill the 4th instars as a contact poison at 5.8, 99.2, and 39.19 mg [AI]/L, respectively (Table 4). Only abamectin and acetamiprid were highly toxic to the pupae of ladybirds, at below 0.09 and 5.00 mg [AI]/L, respectively. In addition, these two compounds, and pyrazophos, were very toxic to adult ladybirds (Table 4).

4. Discussion

For integrated pest management of aphids in the greenhouse, the susceptibility of natural enemies to pesticides must be examined, as well as pesticide toxicity against aphids. Several methods have been used for estimating pesticide compatibility with biological control agents. Beneficial arthropods can be exposed to pesticides by direct contact, by indirect contact with residues on plant surfaces, or by the ingestion of pesticide-contaminated prey or hosts (Jepson, 1989). In most studies, pesticide impact has been evaluated by exposure of the natural enemy to a range of pesticide concentrations (Stark and Wennergren, 1995; Stark et al., 1995; Su et al., 1987; Wiles and Jepson, 1992; Yokoyama et al., 1984). Evaluation of direct toxic effects may include either determining the percentage of dead individuals at a fixed time (LD_{50}) or recording the course of mortality over time (LT_{50}). A highly toxic compound applied at a low rate in the field may cause less mortality than a less toxic compound used at a higher rate. For this reason, it may be more useful to compare the relative toxicity of insecticides to natural enemies by measuring the LC_{50}. In addition, the responses of different life stages have been studied to estimate the effects upon population levels (Stark et al., 1995).

This study showed different contact toxicities for 12 pesticides against different stages of *H. axyridis* when applied at dosages recommended for field use and calculated by LC_{50} values. Toxicities of the four insecticides (acetamiprid, etofenprox, imidacloprid, and thiamethoxam), an acaricide (abamectin) and a fungicide (pyrazophos) varied.

Over the last few years, acetamiprid and imidacloprid have been key compounds in IPM in greenhouse vegetable crops when no efficient biological control agents against aphids were available. Even when predators are available, these two insecticides are still commonly used for aphid and thrips control. Direct exposure to imidacloprid caused significant mortality to eggs and early instar *H. axyridis*. Our study suggests that only 25% of the 3rd instar and none of the eggs, 1st and 2nd instar would survive an application at the recommended concentration. The high toxicity of imidacloprid for younger stages of *H. axyridis* indicates that the foliar spray application of this chemical must be considered carefully for use in IPM programs.

Additional mortality may occur through residual toxicity or by feeding on treated aphids. De Cock et al. (1996) suggested, with the predatory stinkbug *Podisus maculiventris* (Say), that soil applications or seed dressings might reduce mortality. This compound is also moderately persistent for the predatory insects *Dicyphus tamaninii* (Wagner) and *Macrolophus caliginosus* Wagner (Faguls et al., 1999). Imidacloprid was also quite toxic to some heteropteran predators such as *Orius laevigatus* (Fieber) and *P. maculiventris* when exposed to fresh residue (De Cock et al., 1996; Delbeke et al., 1997). When tested under field conditions, it was harmful to *Geocoris punctipes* (Say) for 1–3 days after application (Boyd and Boethel, 1998). Imidacloprid appears to be relatively compatible with the use of the parasitic wasp *Encarsia formosa* for controlling whiteflies (Bethek and Redak, 1997). In addition, imidacloprid could be mediated enhancement of the fecundity and population development of *Amblyseius victoriensis* Womersley, a predatory mite attacking a pest mite (James, 1997; James and Vogele, 2001). The four insecticides could be divided into two classes: one is very deleterious (e.g., acetamiprid), while the other is safe to the pupae of ladybirds (e.g., etofenprox, imidacloprid, and thiamethoxam).

The bacterial fermentation product abamectin was very toxic to *H. axyridis* as well as to *Orius laevigatus* (Fieber), a generalist predator of mites, thrips, and aphids including western flower thrips, *Frankliniella occidentalis* (Pergande) (Van de Veire et al., 1996). Abamectin was the most toxic pesticide in the current study, but had a lower application rate and, consequently, a higher LC_{50} field rate ratio than the other pesticides. Chlorfenapyr was also found to result in a 60–80% survival rate with adults at producer recommended concentrations.

Other than pyrazophos, the acaricides and fungicides were non-toxic to larval and adult *H. axyridis* and are, therefore, more selective towards the ladybird than the insecticides. The acaricides and pyrazophos caused some mortality to eggs, but along with the other fungicides tested here can be considered reasonably safe for use in greenhouse IPM.

Many predators exposed to more than one compound suffer synergistic detrimental effects, even for compounds that were equitably harmless when tested separately (Petersen, 1993). However, we cannot speculate from our data as to potential damaging effects of pesticide combinations. Moreover, selectivity of pesticides for IPM cannot be assessed by laboratory tests alone. The IOBC Working Group has assumed that pesticides found harmless to a specific beneficial insect in laboratory tests are likely to be of low risk to the populations in the field (Bigler and Waldburger, 1994). Our data suggest that some pesticides are compatible with current IPM systems.

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