COMPARISON OF THREE DIFFERENT LABORATORY AND ONE SEMI-FIELD TEST METHODS TO ASSES THE SIDE EFFECTS OF PESTICIDES ON Trichogramma cacoeciae

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ABSTRACT

The side effects of 139 pesticides on **Trichogramma cacoeciae** using three different types of laboratory test methods was studied. The combination of tests used included: (a) initial toxicity test on adult parasites (susceptible life stage), (b) direct spray of parasites pupa within host eggs (less susceptible life stage) and (c) duration of harmful activity (persistence) on adults. The combination was chosen to include two different developmental stages of the natural enemy that greatly vary in their susceptibility as well as in their vulnerability to pesticides. The persistent test show the duration of the harmful activity and help to asses the impact of the chemical. In addition, the initial toxicity of 9 pesticides on **Trichogramma** adults was compared using laboratory and semi-field tests.

The results showed that the preparations greatly differ in their initial toxicity as well as in their persistence. 23 insecticides/acaricides, 10 fungicides, 7 herbicides and plant growthregultors were harmful to the adult parasite but harmless to moderately harmful to the **Trichogramma**-pupa within the host eggs. 13 insecticides/acaricides, 5 fungicides, 6 herbicides and plant growthregultors were harmful in the initial toxicity test but were short-lived to moderately persistent and therefore are much more useful for use in integrated control. Short lived preparations are likely to have much less impact on the natural enemy than persistent ones. The experiments to compare the initial toxicity in laboratory and semi-fields tests showed very little differences. This can be attributed to the similar mode of exposure in these tests.

INTRODUCTION

A combination of several standard laboratory tests together with semi-field and field methods was recommended by the Working Group "Pesticides and Beneficial Organisms" of the International Organization for Biological Control (IOBC), West Palearctic Regional Section (WPRS) to asses the side effects of pesticides on beneficial arthropods. The methods used in the tests were published by HASSAN 1977, 1980 and 1992. The present study aims to evaluate the role of three different types of laboratory test methods in assessing the side effects on **Trichogramma cacoeciae.** The comparison is also an attempt to interpret the results of the different types of tests.

MATERIALS AND METHODS

The experiments were carried out using standard IOBC/WPRS methods within the 6 joint pesticide testing programs of the Working Group. The preparations and the concentrations tested are given in Table 1 - 3.

(a). **Laboratory, susceptible life stage** (adults of parasites):

The initial toxicity was tested by exposing the adult parasites to a fresh dry pesticide film applied on glass plates at the recommended concentration. The exposure cage consisted of two square glass plates and an aluminum frame (13 cm long, 1.5 cm high and 1 cm wide). Each of three sides of the frame contained 6 ventilation holes (1 cm diameter), covered with black tight material. Two portable opening on the fourth side of the frame were used to introduce the **Thichogramma**, host eggs and food. The cage was held together with two clamps. The glass plates were sprayed with the pesticide at the recommended concentration as indicated in Table 1. The experiment started with a 24 hrs period of forced exposure. At the end of the 24 h exposure, the parasites, if still alive, were given host eggs to measure their parasitization capacity. Eggs of the Angoumois grain moth **Sitotroga cerealella** (Oliv.) were offered on the 2, 3 and 5 day of the experiment. The capacity of parasitism per **Trichogramma**-adult female and the reduction in capacity compared with control (treated with water) was used to measure the effect of the chemical. The pesticides were then classified in four categories as shown in Table 1.

(b). **Laboratory, less susceptible life stage** (parasites within their hosts):

Seven day old T. cacoeciae pupae within **Sitotroga**-eggs were directly sprayed and the emerging parasites, if any, were tested for their capacity to parasitize host eggs. The same experimental cage but with untreated glass plates and the method of assessment of parasitism described above were used.

(c). **Laboratory, duration of harmful activity** (persistence toxicity on adults):

The technique used to test persistence of pesticide residues involves the spraying of potted vine plants, maintaining them under field or field simulated environment and exposing adult **Trichogramma** to samples of the treated leaves, taken at different time intervals after application. Exposure tests were carried out 3, 10, 17, 31 days after the treatment of the vine plants. The same experimental cage as in (a), using untreated glass was utilized. The sampled leaves were spread inside the cage to the entire lower surface. The reduction in parasitism compared to was plotted on a probit scale against time. The persistence is the time required for the pesticide residue to loose effectiveness so that a reduction in parasitism of less than 50%, compared with the control is reached.

(d) **Semi-field test** (adults of parasites):

The semi-field method involved the spraying potted apple tree, enclosing the crown of the treated tree in a cage (80 x 80 x 80 cm) consisted of a metal frame with material walls and releasing of 24 hours old adult parasites. Stripes od paper of paper with honey and agar were placing on the foliage. The natural enemy was exposed to the treated tree for a period of one day before the assessment of survival was started. Assessment was carried out by distributed stripes of paper on the foliage with eggs of the Angoumois grain moth **Sitotroga cerealella** (Oliv.) glued on them. About 5000 **Trichogramma** adults were released per cage and 5000 host eggs were added on the second, third and fifth day after the beginning of the exposure (a total of 1500 eggs per cage). The number of host eggs parasitized during the course of the experiment is counted at least 9 days after parasitism. The reduction in parasitism compared to water treated trees was assessed and the pesticides were classified according to the categories given in Table 1 (DICKLER, HASSAN 1979, HASSAN 1977 & 1992).

RESULTS AND DISCUSSION

The laboratory tests:

The results of testing 60 insecticides / acaricides (Table 1), 45 fungicides (table 2), 34 herbicides / plant growth regulators (Table 3) on **Trichogramma** using the tree different methods showed that the chemicals differed markedly in their initial as well as their residual toxicity. In each Table, the preparations were listed according to their increasing toxicity in the initial contact test. In each evaluation category, the duration of harmful activity (persistent) was considered to be more important than effect of the preparation on the parasite within its host (less susceptible life stage).

Short lived preparations are much more suitable for use in modern plant protection. Persistent chemicals affect natural enemies for periods of time and are therefore likely to have a much greater impact on the natural enemy in the field.

The semi-field test

Comparison between the results of the laboratory and the semi-field experiments using the 9 pesticides are given in Table 4. The two insecticides Dimilin (diflubenzuron) and Zoecon 619 and the fungicide Aspor-C (zineb) were harmless / slightly harmful to **Trichogramma** both in the laboratory and in the semi-field tests. Kelthane (dicofol) was moderately harmful to the parasite in both types of tests. Despirol (kelevan) was moderately harmful in the laboratory but harmful in the semi-field test. Plictran 25 W (cyhexatin), Afugan (pyrazophos), Torak (dialifos) and Rubitox WP (phosalon) were harmful in the laboratory as well as in the semi-field test.

Table 1: Results of three different laboratory tests on **Trichogramma** cacoeciae
(1) initial toxicity on adults (susceptible life stage),
(2) Pupae within Sitotroga-eggs (less susceptible life stage) and
(3) persistence toxicity on adults (duration of harmful activity).

Preparation (Active ingredient)	Conc. testes %	Adult	Pupa within host egg	Persis-tence
Insecticides / acaricides				
1 Dipel (Bacillus thuringiensis)	0.10	1	-	-
2 Torque (fenbutation-oxid)	0.05	1	-	-
3 Dimilin (diflubenzuron)	0.05	1	-	-
4 Apollo SOSC (clofentezine)	0.04	1	-	-
5 Cesar (hexythiazox)	0.025	1	-	-
6 Insegar (fenoxycarb)	0.06	1	-	-
7 Applaud (buprofezin)	0.03	1	-	-
8 Dimilin (diflubenzuron)	0.05	1	-	-
9 Trigard (cyromazine)	0.067	1	-	-
10 Neudosan (Kali-Seife)	2.0	1	-	-
11 Delfin WG (Bacillus thuring.)	0.10	1	-	-
12 Novador FC (Bac.thuring.tenebr.)	0.50	1	-	-
13 Micro Germin (Verticillium lec.)	0.2	1	-	-
14 Nomolt (teflubenzuron)	0.10	1	1	-
15 Azomate (benzoximate)	0.15	2	-	-
16 Kelthane (dicofol)	0.15	3	1	2
17 Evisect S (thiocyclam)	0.03	3	3	2
18 Cropotex (flubenzimine)	0.10	3	1	4
19 Pirimor-Granulat (pirimicarb)	0.10	4	1	1
20 Croneton (ethiophencarb)	0.10	4	1	2
21 Tedion V 18 (tetradifon)	0.20	4	1	2
22 Asepta Lindane (lindane)	0.10	4	3	2
23 Dimecron 20 (phosphamidon)	0.25	4	3	2

24	Spruzit-Nova-flussig(pyrethrum+)	0.10	4	4	3
25	Unden (propoxur)	0.15	4	4	2
26	Basudine vloeibar (diazinon)	0.21	4	4	2
27	Phosdrine W 10 (mevinphos)	0.58	4	4	2
28	Dipterex WP 80 (trichlorphon)	0.10	4	2	3
29	Thiodan 35 Spritzp. (endosulfan)	0.10	4	3	3
30	Hostaquick (heptenophos)	0.10	4	4	3
31	Peropal (azocyclotin)	0.10	4	1	3
32	Plictran 25 W (cyhexatin)	0.10	4	1	4
33	Rubitox Spritzp.(phosalone)	0.20	4	1	4
34	Ambush (permethrin)	0.02	4	1	4
35	Orthen (acephate)	0.15	4	2	4
36	Mitac (amitraz)	0.30	4	2	4
37	Decis (deltamethrin)	0.06	4	2	4
38	Gusathion (azinphos-methyl)	0.20	4	3	4
39	Kilval (vamidothion)	0.125	4	1	4
40	Vydate L (oxamyl)	0.15	4	1	4
41	Rody (fenpropathrin)	0.05	4	1	4
42	Klartan (fluvalinate)	0.06	4	1	4
43	Baythroid 50 (cyfluthrin)	0.05	4	2	4
44	Karate (lambda-cyhalothrin)	0.075	4	2	4
45	Tamaron (methamidophos)	0.15	4	4	4
46	Torak E (dialiphos)	0.25	4	3	4
47	Lannate (methomyl)	0.10	4	4	4
48	Sumicidin (fenvalerate)	0.075	4	4	4
49	Actellic 50 (pirimiphos-methyl)	0.20	4	4	4
50	Ultracid (methidathion)	0.075	4	4	4
51	Folithion (fenitrothion)	0.10	4	4	4
52	Hostaquick (heptenophos)	0.10	4	4	4
53	Ekamet (etrimfos)	0.20	4	4	4
54	Asepta Nexion (bromophos)	0.27	4	4	4
55	Birlane EC 40 (chlorfenvinphos)	0.33	4	4	4

56	Dursban Spritzp. (chlorpyfifos)	0.25	4	4	4
57	Ambush C (cypermethrin)	0.04	4	4	4
58	Perfekthion (dimethoate)	0.21	4	4	4
59	Hostathion (triazophos)	0.24	4	4	4
60	Imidan (phosmet)	0.25	4	2	-

Initial toxicity: 1 = harmless (<30%>,
2 = slightly harmful (30-79%),
3 = moderately harmful (80-99%),
4 = harmful (>99%).

Persistence: 1 = short-lived,
2 = slightly persistent,
3 = moderately persistent,
4 = persistent.

Table 2: Results of three different laboratory tests on **Trichogramma cacoeciae**
(1)initial toxicity on adults (susceptible life stage),
(2)pupae within **Sitotroga**-eggs (less susceptible life stage) and
(3)persistence toxicity on adults (duration of harmful activity).

Preparation (Active ingredient)		Conc. tested %	Adult	Pupa Within host egg	Persistence
F u n g i c i d e s (Active ingredient)		Tested		Within	Tence
1	Nimrod (bupirimate)	0.04	1	-	-
2	Cercobin-M(thiophanat-methyl)	0.10	1	-	-
3	Ortho Difolatan (captafol)	0.20	1	-	-
4	Orthocid 83 (captan)	0.15	1	-	-
5	Bayleton (triadimefon)	0.10	1	-	-
6	Ronilan (vinclozolin)	0.05	1	-	-
7	Derosal (carbendazim)	0.05	1	-	-
8	Daconil 500 (chlorothalonil)	0.30	1	-	-
9	Milgo-E (ethirimol)	0.18	1	-	-
10	Ortho-Phaltan 50 (folpet)	0.33	1	-	-

11	Topas (penconazole)	0.04	1	-	-
12	Baycor (bitertanol)	0.37	1	-	-
13	Delan flussig (dithianon)	0.20	1	-	-
14	Vitigran (copper-oxychlorid)	1.00	1	-	-
15	Impact	0.16	1	-	-
16	Rovral PM (iprodion)	0.15	1	-	-
17	Saprol (triforine)	0.15	1	-	-
18	Sumisclex (procymidone)	0.15	1	-	-
19	Dyrene flussig (anizaline)	0.40	1	-	-
20	Bayfidan (triadimenol)	0.05	1	-	-
21	Anvil (hexaconazole)	0.03	1	-	-
22	Calixin (tridemorph)	0.075	1	-	-
23	Alto 100 SL (cyproconazol)	0.08	1	-	-
24	Score EC 250 (difenoconazol)	0.05	1	-	-
25	BioBlatt Mehltaumittel (lecithin)	0.15	1	-	-
26	Dithane Ultra (mancozed)	0.10	2	-	-
27	Pormarsol forte (thiram)	0.20	2	-	-
28	Rubigan Vloeibaar (fenarimol)	0.12	2	-	-
29	Antracol (propineb)	0.20	2	-	-
30	Omnex WP 10 (penconazol)	0.025	2	-	-
31	Tilt (propiconazole)	0.08	3	1	1
32	Dithane Ultra (mancozeb)	0.20	3	1	2
33	Trimidal EC (nuarimol)	0.08	3	1	2
34	Plondrel (ditalimfos)	0.075	3	1	3
35	Netzschwefel Bayer (sulphur)	0.40	3	1	4
36	Corbel (fenpropimorph)	0.17	4	1	1
37	Euparen (dichlofluanid)	0.20	4	1	3
38	Sportak (prochloraz)	0.187	4	1	3
39	Euparen (dichlofluanid)	0.15	4	1	3
40	Nevikén (lime-sulphur)	3.00	4	2	3
41	Polyram-Combi (metiram)	0.42	4	1	4
42	Afugan WP 30 (pyrazophos)	0.05	4	1	4

43	Triovit (sulphur)	0.40	4	1	4
44	Morestan (chinomethionate)	0.10	4	1	4
45	Dithane M 22 (maneb)	0.50	4	1	4

Initial toxicity: 1 = harmless (<30%),
2 = slightly harmful (30-79%),
3 = moderately harmful (80-99%),
4 = harmful (>99%).

Persistence: 1 = short-lived,
2 = slightly persistent,
3 = moderately persistent
4 = persistent.

Table 3: Results of three different laboratory tests on **Trichogramma cacoeciae**
(1) initial toxicity on adults (susceptible life stage),
(2) pupae within **Sitotroga**-eggs (less susceptible life stage) and
(3) persistence toxicity on adults (duration of harmful activity)

Preparation (Active ingredient)		Conc. tested %	Adult	Pupa within host egg	Persis-tence
Herbicides / Plant growth regultors					
1	Betanal (phenmedipham)	2.25	1	-	-
2	Hyvar X (bromacil)	0.20	1	-	-
3	Gesatop 50 (simazin)	0.375	1	-	-
4	Fusilade (fluazifop-butyl)	0.25	1	-	-
5	Luxan 2,4-D amine (aminesalt)	0.432	1	-	-
6	Tribunil (metabenzthiazuron)	0.67	1	-	-
7	Ally (metsulfuron-methyl)	0.076	1	-	-
8	Dirigol-N (alphanaphthyl-acetamid)	0.02	1	-	-
9	Exp.30004 A (ioxynil)	0.24	1	-	-
10	Lontrel 100 (clopyralid)	0.12	1	-	-
11	Targa (quizalofop-ethyl)	0.30	1	-	-
12	Grasp (tralkoxydim)	0.50	1	-	-

13	Basagran (bentazone)	0.40	1	-	-
14	Tramat 500 (ethofumesat)	1.00	1	-	-
15	Starane 180 (fluroxypyr)	0.50	1	-	-
16	Arelon flussig (isoproturon)	0.75	1	-	-
17	Goltix 70 WG (metamitron)	2.50	1	-	-
18	Illoxan (diclofop- methyl)	0.75	2	-	-
19	Ustinex PA (amitrol+diuron)	1.00	2	-	-
20	Gesaprim 50 (atrazin)	0.67	2	-	-
21	Basta (glufosinate-ammonium)	0.50	2	-	-
22	Roundup (glyphosate)	1.00	2	-	-
23	Faneron (bromofenoxim)	1.70	3	1	-
24	Gallant Super (haloxyfop)	0.50	3	1	1
25	Semeron (desmetryne)	0.25	4	1	2
26	Cycocel Extra (chlormequat)	0.70	3	1	3
27	Kerb 50 W (propyzamid)	0.75	3	1	4
28	Fervinal Plus (sethoxydim)	0.79	4	1	1
29	Avange (difenzoquat)	1.00	4	1	2
30	Rhodofix (1-naphthyl-acetic acid)	0.15	4	1	2
31	Certrol B (bromoxynil)	0.33	4	1	2
32	Ramrod (propachlor)	1.00	4	3	2
33	Aretit flussig (dinoseb)	1.25	4	4	4
34	Prosevor (carbaryl)	0.125	4	4	4
35	Aresin (monolinuron)	0.75	4	2	-

Initial toxicity:
1 = harmless (<30%),
2 = slightly harmful (30-79%),
3 = moderately harmful (80-99%),
4 = harmful (>99%).

Persistence:
1 = short-lived,
2 = slightly persistent,
3 = moderately persistent,
4 = persistent.

Table 4:Comparison of laboratory and semi-field results (**Trichogramma cacoeciae**), tests carried out by DICKLER and HASSAN.

Pesticide (Category) (Activite ingr.)	Conc. %	Reduction in parasitization in % laboratory		semi - field	
Dimilin (diflubenzuron)	0.05	32.1	(1)	0	(1)
Zoecon 619 *	0.10	71.6	(2)	0	(1)
Aspor-C * (zineb)	0.20	5.9	(1)	13.1	(1)
Kelthane * (dicofol)	0.15	96.3	(3)	60.0	(3)
Plictran 25 W*(cyhexation)	0.10	100	(4)	83.1	(4)
Despirol * (kelevan)	0.05	97.0	(3)	87.0	(4)
Afugan (pyrazophos)	0.05	100	(4)	89.6	(4)
Torak * (dialifos)	0.10	100	(4)	92.1	(4)
Rubitox WP (phosalon)	0.20	100	(4)	93.4	(4)

Laboratory:
1 = harmless (<30%),
2 = slightly harmful (30-79%),
3 = moderately harmful (80-99%),
4 = harmful (>99%).

Semi-field:
1 = harmless (<25%),
2 = slightly harmful (25-50%),
3 = moderately harmful (51-75%),
4 = harmful (>75%).

* not registered in Germany at this time.

The results indicated that differences between the results the results of the two types of tests were found only with two out of a total of nine pesticides. In both cases, the toxixity was higher in the laboratory than in the semi-field test. However, the difference in both cases was only with one category.

The differences in the results of the laboratory and semi-field tests were smaller than espected. This can be attributed to the similar mode of exposure in the two methods. In both cases, the adults parasites were exposed to a fresh dry pesticide film. The differences could be partly due to the nature of the exposure surface and the size of the exposure larger cage. The chemicals

were applied on glass plates in the laboratory and on apple foliafe in the semi-field. The size of the laboratory cage was 13 x 13 x 1,5 cm, the semi-field 80 x 80 x 80 cm. To improve the laboratory method, the air in the cage was continuously changed to prevent the accumulation of pesticides fumes. Each cage was connected to aguarium pump through a tube system. The entire air in the cage was exchanged every 1 to 2 minutes.

LITERATURE

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