

**EFFECTS OF BOTANICAL INSECTICIDES ON THE EGG
PARASITOID *TRICHOGRAMMA CACOECIAE* MARCHAL
(HYM. TRICHOGRAMMATIDAE).**

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Abstract

Laboratory studies were carried to investigate the side effects on *Trichogramma cacoeciae* of two formulated products of each of two botanical insecticides: Azadirachtine (Neemazal T/S Blank and Celaflo®) and Quassin (alcoholic or water extracts). The results showed that by exposing adults *T. cacoeciae* to residues of Neemazal formulations on glass plates, the tested preparations were either harmful (Neemazal-Blank) or moderately harmful (Celaflo). The two Quassin formulations tested were harmless. When treated host eggs were offered to adults *T. cacoeciae*, all tested chemicals were almost harmless. In a further test, host eggs parasitized at different time intervals (1-8 days), were sprayed at the same day. The results indicated that only Neemazal T/S-Blank formulation was slightly to moderately harmful reducing adult emergence.

Keywords: Side effects, Trichogramma, Botanical insecticides, Neemazal, Quassin.

Introduction

Parasitoids of the genus *Trichogramma* occur naturally worldwide and play an important role as natural enemies of lepidopterous pests on a wide range of agricultural crops. During the past two decades *Trichogramma* wasps have been used as biological control agents for pest suppression. Results of augmentative releases of *Trichogramma* can be affected by the use of broad-spectrum insecticides in or near release plots (Stinners *et al.* 1974, Ables *et al.* 1979, King *et al.* 1984). The search for selective insecticides to be used with *Trichogramma* releases is of great importance. The recent laboratory studies were carried out to investigate the side effects on *Trichogramma cacoeciae* of two formulated products of each of two botanical insecticides: Azadirachtine (Neemazal T/S Blank and Celaflo®) and Quassin (alcoholic or water extracts) to study their possible use with *Trichogramma* releases, since these insecticides are coming from plant origin they are believed also to have the advantage of having the least impact on the environment.

Materials and methods

Test Insecticides Two formulations of the botanical active ingredient, azadirachtine (Neemazal T/s Blank and Celaflo) as well as two extracts of Quassin (Alcoholic and Water extracts) were included in the tests. The field recommended concentrations were prepared for the tests.

a. Susceptible life stage (adults of parasites):

1- Exposing adults of Trichogramma to sprayed glass plates:

The initial toxicity was tested by exposing the adult parasites to a fresh dry pesticide film applied on glass plates at the highest recommended field rate. The method described by Hassan et al. (2000) was followed. The exposure cage consisted of two square glass plates and an aluminium 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 *Trichogramma*, host eggs and food. The cage was held together with two clamps. The glass plates were sprayed with the pesticide at the recommended field rates. The experiment started with a 24 h period of forced exposure. At the end of the 24 h exposure, the parasites were given host eggs to measure their capacity of parasitism. 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 the control (treated with water) was used to measure the effect of the chemical. The pesticides were then classified in four categories based on the IOBC classification (Hassan et al. 2000).

2-Exposing adults of Trichogramma to sprayed host eggs:

In another experiment adult *Trichogramma* were offered sprayed host eggs for parasitisations. The treated host eggs were either offered directly after drying of the spray or the eggs were hold at 15 °C and offered to adults after 6 days. In these experiments, the adults were anthesised for short period (ca. 45 seconds) with CO₂ and put onto treated host eggs. The start population was then determined through direct counts. The parasitism rate per female was calculated and the reduction in parasitism relative to the control was then determined. The tested insecticides were classified accordingly.

b-Less susceptible life stage (parasites within their hosts):

The method described by Hassan and Abdelgader (2001) was followed. Accordingly seven day old *T. cacoeciae* pupae within *Sitotroga*-eggs were directly sprayed with the same insecticides and concentrations used during the adults tests. The study included spraying of parasitised host eggs at different interval after parasitisation ranging from 1 – 8 days. The percentage of adult emergence and the reduction in emergence relative to the control were then determined and the pesticides were categorized accordingly.

Results and discussion:

Effects on adults

Results of tested Botanicals on adults are presented in Table (1). The results showed that by exposing adults *T. cacoeciae* to residues of Neemazal formulations on glass plates (standard test method, Hassan et al. 2000), the preparations were either harmful (Neemazal-Blank) or moderately harmful (Celaflor). The two Quassin formulations tested were harmless. In another set of experiments, where treated host eggs were offered to adults *T. cacoeciae*, all tested chemicals were almost harmless.

By exposing adults to treated host eggs both Quassin formulations were harmless. Celaflor was slightly toxic for adults, both when freshly or 6-day old sprayed host eggs were offered to adults of *T. cacoeciae*. Neemazal-Blank formulation was only slightly toxic when 6 day old sprayed host eggs were offered to the adults.

Table 1. Effects of exposing adult *Trichogramma cacoeciae* to treated glass plates on rate of parasitism (Initial Laboratory adults Test).

Trtreatment	Reps	eggs/female	**	SE	% RC	Class
Control	4	18.87	abc	4.8		
Quassin-Alcohol	4	21.22	bc	4.8	-12.5	1
Quassin-Water	4	21.59	c	4.8	-14.4	1
Neemazal-Blank	4	0.00	a	4.8	100	4
Celaflor	4	0.96	ab	4.8	94.89	3

** = Figures followed by the same letter are not significantly different (Multiple Range Test , 5%)

SE = Standard Error

% RC = Percentage Reduction relative to the control

Class = IOBC classification

Table (2). Effects of exposing adult *Trichogramma cacoeciae* to treated eggs on rate of parasitism.

Treatment	Reps	Fresh Residues					6 day old Residues				
		eggs/ female	**	SE	% R	Class	eggs/ female	**	SE	% R	Class
Control	4	28.84	bc	2.0			36	b	3.1		
Quassin-Alcohol	4	23.11	ab	2.0	19.88	1	31.63	ab	3.1	12.15	1
Quassin-Water	4	33	c	2.0	-14.4	1	33.9	b	3.1	5.83	1
Neemazal-Blank	4	24.01	ab	2.0	16.75	1	23.98	a	3.1	33.4	2
Celaflor	4	20.33	a	2.0	29.5	1-2	23.15	a	3.1	35.69	2

** = Figures followed by the same letter are not significantly different (Multiple Range Test , 5%)

SE = Standard Error

% RC = Percentage Reduction relative to the control

Class = IOBC classification

Effects on immature stages

Results of spraying parasitized host eggs at different time intervals (1-8 days) are presented in Table 3 and 4.

Spraying parasitised host eggs one day after parasitism resulted in a significantly lower number of black eggs (i.e. lower pupation), when host eggs were sprayed one day after parasitism with Azadirachtine formulation. The reduction in pupation caused by Neemazal T/S formulation was more pronounced. All tested insecticides significantly reduced pupation, when host eggs were sprayed two days after parasitism, indicating that *Trichogramma* was very sensitive during this stage. This might have coincided with the hatching of the vulnerable neonate larvae of *Trichogramma* from laid eggs. The pupation rate was not reduced as a result of treatment, when host eggs were sprayed on the third and subsequent days after parasitism (Table 3 a). This trend can also be seen clearly when the percentage reduction relative to the control and the categorisation according to the IOBC classification was determined (Table 3b).

In regard to the percentage developing adults from larvae reaching pupation, Neemazal formulation consistently resulted in significantly lower percentage of developing females. The other azadirachtine formulation (Celaflor) resulted in significantly lower percentage of emerging adults, when the eggs were sprayed one day after parasitism (Table 4 a). However, based of the IOBC classification Neemazal T/S was slightly toxic for immature stages, when parasitised host eggs were sprayed after the third day or afterwards (Table 4b)

Table 3a. Developing Black eggs after treating parasitised eggs at various days after parasitism

Treatment	Count	1 day			2 days			3 days			5 days			7 days			8 days		
		Mean	**	SE	Mean	**	SE	Mean	**	SE	Mean	**	SE	Mean	*	SE	Mean	**	SE
Control	6	427.33	c	17.45	329.00	a	20.85	388.3	ab	19.3	465.17	ab	26.04	440.17	b	19.45	355.50	ab	20.79
Quassin-Alcohol	6	400.83	c	17.45	189.83	b	20.85	441.7	bc	19.3	464.17	a	26.04	420.67	b	19.45	388.50	bc	20.79
Quassin-Water	6	401.67	c	17.45	247.83	b	20.85	448.8	c	19.3	506.33	b	26.04	412.00	b	19.45	421.33	c	20.79
Neemazal-Blank	6	219.00	a	17.45	219.83	b	20.85	357.5	a	19.3	437.50	a	26.04	340.33	a	19.45	325.33	a	20.79
Celaflor	6	334.33	b	17.45	197.00	b	20.85	466.5	c	19.3	430.17	a	26.04	420.00	b	19.45	323.17	a	20.79

** = Figures followed by the same letter are not significantly different (Multiple Range Test , 5%)

SE = Standard Error

Table 3b. Developing Black eggs after treating parasitised eggs at various days after parasitism (IOBC – Classification)

Treatment	Reps	1 day		2 days		3 days		5 days		7 days		8 days	
		% RC	Class	% RC	Class	% RC	Class	% RC	Class	% RC	Class	% RC	Class
Control	6												
Quassin-Alcohol	6	6.20	1	42.30	2	-13.73	1	0.21	1	4.43	1	-9.28	1
Quassin-Water	6	6.01	1	24.67	1	-15.58	1	-8.85	1	6.40	1	-18.52	1
Neemazal-Blank	6	48.75	2	33.18	2	7.94	1	5.95	1	22.68	1	8.49	1
Celaflor	6	21.76	1	40.12	2	-20.13	1	7.52	1	4.58	1	9.10	1

% RC = Percentage Reduction relative to the control

Class = IOBC classification

Table 4 a. Percentage of developing adults after treating parasitised eggs at various days after parasitism

Treatment	Count	1 day		2 days		3 days		5 days		7 days		8 days	
		Mean	** SE	Mean	**SE	Mean	** SE	Mean	** SE	Mean	** SE	Mean	**SE
Control	6	89.05	b 2.46	91.81	b 3.27	97.14	c 3.54	95.67	bc 3.07	97.97	b 2.89	91.25	b 3.50
Quassin-Alcohol	6	86.38	b 2.46	95.13	b 3.27	91.03	bc 3.54	93.61	bc 3.07	97.52	b 2.89	90.42	b 3.50
Quassin-Water	6	91.02	b 2.46	91.78	b 3.27	96.12	c 3.54	98.22	c 3.07	94.20	b 2.89	87.58	b 3.50
Neemazal-Blank	6	77.78	a 2.46	66.57	a 3.27	52.55	a 3.54	50.51	a 3.07	57.33	a 2.89	56.60	a 3.50
Celaflor	6	75.10	a 2.46	92.96	b 3.27	80.73	b 3.54	89.28	b 3.07	91.64	b 2.89	92.70	b 3.50

** = Figures followed by the same letter are not significantly different (Multiple Range Test , 5%)
SE = Standard Error

Table 4 b. Percentage of developing adults after treating parasitised eggs at various days after parasitism

Treatment	Reps	1 day		2 days		3 days		5 days		7 days		8 days	
		% RC	Class	% RC	Class	% RC	Class	% RC	Class	% RC	Class	% RC	Class
Control	6												
Quassin-Alcohol	6	3.00	1	-3.61	1	6.29	1	2.15	1	0.46	1	0.92	1
Quassin-Water	6	-2.20	1	0.04	1	1.05	1	-2.66	1	3.85	1	4.03	1
Neemazal-Blank	6	12.66	1	27.50	1	45.91	2	47.21	2	41.48	2	37.97	2
Celaflor	6	15.67	1	-1.25	1	16.90	1	6.68	1	6.46	1	-1.59	1

% RC = Percentage Reduction relative to the control
Class = IOBC classification

Conclusion

The results showed, in general, that both Azadirachtine and Quassin were relatively safe to the tested parasitoid and could therefore be used in combination with *Trichogramma* releases.

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